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THE BIOCHEMISTRY OF SYNTHESIS IN ACTIVATED SLUDGE

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THE BIOCHEMISTRY OF SYNTHESIS IN ACTIVATED SLUDGE

James M. Symons
Project Director

April 1963

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THE BIOCHEMISTRY OF SYNTHESIS IN ACTIVATED SLUDGE

by

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SUMMARY

This investigation concerns the behavior of the activated sludge process with respect to the biological solids produced while treating simple, soluble substrates under varying operating conditions. Several small, 0.5 to 2.5 liter, activated sludge pilot plants were operated under various operating situations, and the behavior, especially with respect to the solids resulting from a unit of substrate (COD) removed (Mr/Fr ratio), was noted. Both batch-fed units, simulating conventional activated sludge, and continuously-fed units, simulating complete mixing activated sludge, were studied.

In general, the results of these studies showed that the feeding schedule, batch-fed or continuously-fed, had a strong influence on the resultant solids produced while the solids wasting schedule had little influence, all other things being equal. Substrate has a strong influence on the resultant solids produced, the influence being greatest at short solids retention times. Carbohydrate substrates produced the most solids, short chain fatty acids the least. At longer solids retention times the inert material which accumulates seems to be unrelated to substrate-fed. Also the substrate influence is stronger in conventional activated sludge systems as opposed to complete mixing activated sludge

units. Volumetric organic loading has little, if any, influence on the resultant solids produced. The solids retention time has a strong influence on the resultant solids produced both in conventional as well as complete mixing activated sludge, the longer the solids retention time, the lower the Mr/Fr ratio. The quantity of nitrogen added to a complete mixing activated sludge system as a nutrient has an influence on the resultant solids produced; the less nitrogen added, the greater the Mr/Fr ratio. Also, the less nitrogen which is added to a system, the lower the per cent nitrogen in the volatile solids. The efficiency of COD removal is directly related to this nitrogen level.

In addition to these main studies, several related investigations were made and these are discussed in this report.

I. INTRODUCTION

The ideal biological device for the treatment of waste waters would be one in which all of the organic matter in the influent would be converted to gases or inorganic compounds. Unfortunately, this is not possible since the goal of the microorganism during metabolism is the maximum production of protoplasm (reproduction) with a minimum of influent organic matter being expended for energy. This conversion of influent organic matter into protoplasm is of little benefit to the sanitary engineer since, although it may be removed by settling, it usually must be further treated before final disposal.

Since microorganisms behave differently under varying environmental conditions, it would be reasonable to expect that during varying operating conditions of a biological device, the new protoplasm formed and the resulting biological solids would also vary. These variations are important to define so that the sanitary engineer will know how much organic matter will have to be retreated, that is, solids to be disposed of, for a given set of operating conditions.

This investigation concerns the behavior of the activated sludge process, with respect to the biological solids produced, while treating simple soluble substrates under varying operating conditions.

This report covers the first three years of a planned four-year study. The investigation was terminated one year early due to the resignation of the principal investigator. Since the research was not complete in all phases, some suggestions for future work will be included.

II. OBJECT AND SCOPE

The primary object of this investigation was to determine the effect on the quantity of the biological solids resulting during the metabolism of simple soluble substrates by activated sludge under various operational conditions. The operational conditions covered by this study were:

1. Method of feeding substrate and wasting solids.
2. Nature of substrate.
3. Varying volumetric loading.
4. Varying solids retention time.
5. Quantity and form of nitrogen supplementation.

In addition to this main objective, several other studies were carried out during this project. These will be discussed at the end of this report.

III. DEFINITIONS

For the purpose of this report the following terms will be used as defined below:

1. Organic matter added - Fa - The COD of the influent (soluble substrates used).
2. Organic matter removed - Fr - The change in soluble COD through the system.
3. Solids Created - Mc - The new protoplasm formed during metabolism.
4. Solids Destroyed - Md - The loss in solids due to biological activity.
5. Resultant Solids - Mr - The increase in volatile solids in the mixed liquor plus the volatile solids wasted from the system.
6. Wasted Solids - Mw - The loss in volatile solids from the system due to deliberate removal, in the effluent, and during sampling.
7. Active Mass - Ma - The live microorganisms actually metabolizing the substrate.
8. Mixed Liquor Volatile Solids - Mv - The volatile suspended solids concentration in the aeration tank.
9. Volumetric Loading - Pounds of COD added/1000 ft^3 of Aeration Capacity/Day.
10. Solids Loading - Pounds of COD added/day/pound of Active Mass
11. Fr/Ma Ratio - Pounds of COD removed/day/pound of Active Mass
12. Solids Retention Time - SRT - Volatile solids in the system/volatile solids wasted/day.
13. Conventional Activated Sludge - CAS - An aerobic biological treatment process in which, ideally,

the organic matter in solution and the microorganisms travel together through the aeration tank in a slug. Batch-fed pilot plants biologically simulate this condition.

14. Complete Mixing Activated Sludge - CMAS - An aerobic biological treatment process in which the organic matter added is instantly and completely mixed throughout the entire contents of the aeration tank. Continuously-fed pilot plants biologically simulate this condition.
15. Extended Solids Aeration - Little or no solids wasting, less than three per cent per day with an SRT greater than 30 days.
16. Moderate Solids Aeration - Some solids wasting, between three and thirty per cent per day, SRT between 30 and 3 days.
17. Minimal Solids Aeration - Much solids wasting, more than 30 per cent per day with an SRT less than 3 days.

IV. APPROACH

The approach for the various phases of this investigation are summarized below:

A. Method of Feeding Substrate and Wasting Solids

Three sets of pilot plant pairs were studied. In each set of pairs all conditions were the same, except the methods of feeding and solids wasting. The three sets are summarized below:

Set 1

Complete mixing activated sludge - continuous solids wasting

Conventional activated sludge - batch solids wasting

Set 2

Conventional activated sludge - batch solids wasting

Complete mixing activated sludge - batch solids wasting

Set 3

Complete mixing activated sludge - continuous solids wasting

Complete mixing activated sludge - batch solids wasting

The Mv (mixed liquor volatile solids) levels in the pairs were compared after equilibrium had been reached to determine the effect of the various operational methods.

B. Nature of Substrate

To determine the effect of substrate on the resultant solids in activated sludge systems operated similarly, fifteen conventional activated sludge systems were operated on different soluble substrates. Each system was operated at a 2.5 day SRT and a volumetric loading of 60 lbs COD/1000 cu ft/day. A solids balance for each system provided the measure of the

resultant solids. Three complete mixing systems operated at a loading of 30 lbs COD/1000 cu ft/d at a 200 day SRT metabolizing Na Acetate, glucose, and glycine were compared for resultant solids. Four complete mixing activated sludge systems were operated at an SRT of 10 days and a volumetric loading of 60 lbs COD/1000 cu ft/d and grown Na Acetate, Na Oleate, glucose, and starch were also studied.

C. Varying Volumetric Loading

Two sets of conventional activated sludge systems with volumetric loadings of 30 and 60 lbs COD/1000 cu ft/d were studied. Two complete mixing activated sludge series were operated at different volumetric loadings to determine the effect on the resultant solids. One series had a short SRT, 0.25 days, and volumetric loadings which varied from 60 lbs COD/1000 cu ft/day to 1700 lbs COD/1000 cu ft/day. The other series had a long SRT, 200 days, and had volumetric loadings from 15 to 60 lbs COD/1000 cu ft/day. A solids balance provided the information on the resultant solids. The disposal of the solids from the low SRT systems was also studied.

D. Varying Solids Retention Time

Two series of studies were conducted, one on conventional activated sludge and one with complete mixing activated sludge. Ten conventional activated sludge systems were operated on a once-a-day wasting schedule to give SRT's from 1.3 to 60 days. A solids balance for each system provided the measure of the resultant solids. Similarly, six complete mixing activated sludge systems with SRT's of 1 to 6 days were also compared for resultant solids. In addition, some very short SRT units, 0.25 days, and some very long units, 200 days, were also studied for resultant solids produced per day. Various pure and mixed soluble substrates were used in this study.

E. Quantity and Form of Nitrogen Supplementation

Since nitrogen is an essential element in the formation of new protoplasm, it is quite likely that operating a biological treatment device with less than an optimum quantity of nitrogen will cause changes in the behavior of the sludge. Therefore, several complete mixing activated sludge plants were operated on varying substrates, at varying SRT's, with varying quantities and types of nitrogen. The effect of these changes, in terms of the resultant solids produced and the efficiency of COD removal of the systems, was determined.

V. EQUIPMENT AND PROCEDURES

The pilot plants used in these studies were of 0.5, 1.0, 1.5, 2.0 and 2.5 liters capacity. They were fed on a fill and draw basis or continuously-fed through a low capacity-positive displacement pump. Batch wasting of solids was accomplished by withdrawing a prescribed quantity of mixed liquor each day. Continuous wasting of solids was accomplished by discharging mixed liquor in a volume equal to the liquid quantity of feed added each day. To strive for a representative sample of mixed liquor being discharged, periodically a 50-100 ml slug of mixed liquor was discharged through a dosing syphon. Long SRT systems were operated by employing a settling chamber in the pilot plant to retain most of the mixed liquor solids. The effluent was then centrifuged and the remainder of the solids recycled.

Conventional analytical techniques were employed during this investigation except that the mixed liquor volatile solids were usually determined by the following method. Twenty-five or fifty ml of mixed liquor was centrifuged at 16,000 rpm for several minutes. The clarified supernatant liquor was poured off and 50 ml of distilled water added. This water was then mixed with the solids and the mixture centrifuged again. The clarified liquor was again poured off and the solids washed into a tared crucible. This was then dried overnight at 103°C, weighed, fired at 600°C, cooled and reweighed to obtain the mixed liquor volatile solids. This technique worked very well and was particularly useful in sampling fairly dispersed sludges which would not pass through a membrane filter.

VI. RESULTS

A. Method of Feeding Substrate and Wasting Solids

1. Complete Mixing Activated Sludge-Continuous Solids Wasting (CMAS-CW) vs Conventional Activated Sludge-Batch Solids Wasting (CAS-BW)

In this study two systems were operated on a potassium acetate substrate at a volumetric loading of 60 lbs COD/1000 cu ft/day and a SRT of 6.25 days. One system had the food continuously fed; solids were continuously wasted by continuously discharging mixed liquor (through a dosing syphon) as the food was added. The other system was fed once a day. Just before feeding, 16 per cent of the mixed liquor was siphoned out and Cambridge, Mass., tap water containing the daily feed was added to make up to the original volume. The results of this study are shown in Figure 1. The conventional activated sludge system operated with batch wasting was fed for several days before any mixed liquor volatile solids measurements were made. The mixed liquor volatile solids concentration was significantly higher in the CAS-BW system than in the CMAS-CW system.

2. Conventional Activated Sludge - Batch Solids Wasting (CAS-BW) vs Complete Mixing Activated Sludge - Batch Solids Wasting (CMAS-BW)

These two systems, in which the solids wasting procedure was the same, but the feeding schedule was different, were operated on a nutrient broth, sodium oleate, starch mixture for a substrate. The volumetric loading was 50 lbs COD/1000 cu ft/day and the SRT was 3.7 days. Both systems had all of the discharged solids recycled by centrifugation except the mixed liquor which was wasted each day. The results of this study are shown in Figure 2. After the 21st day of operation the entire contents of the pilot plants were blended in a Waring Blender in an effort to improve the uniformity of wasting and sampling. In spite of the variability of the mixed liquor volatile solids

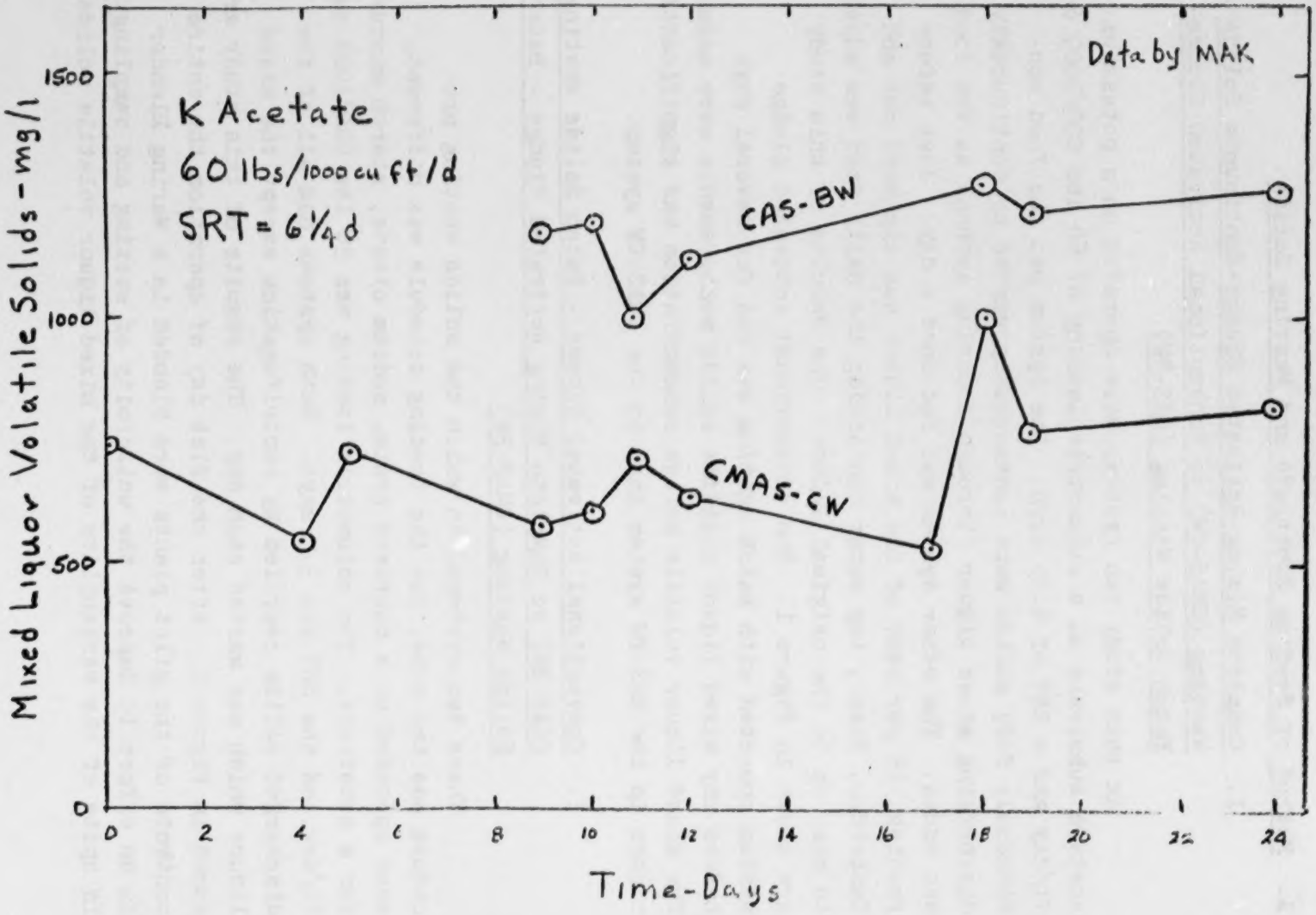

Figure 1 Comparsion of CAS-BW and CMAS-CW

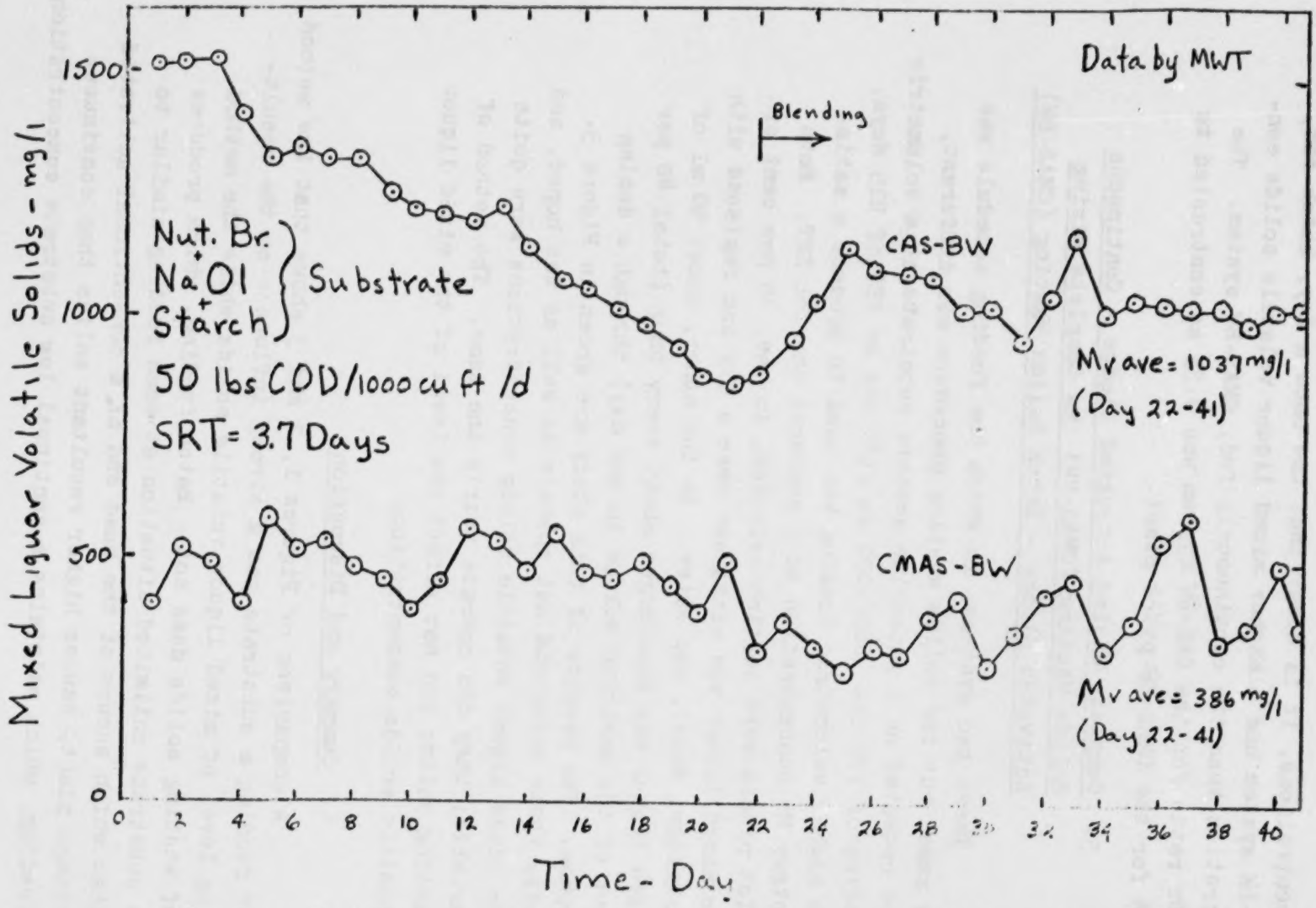

Figure 2 Comparsion of CAS-BW and CMAS-BW

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concentrations, it is clear that the once a day, batch-fed, CAS-BW system had a higher mixed liquor volatile solids concentration than the continuously-fed, CMAS-BW system. The Mr/Fr ratio for the CAS-BW system was 0.35 as contrasted to 0.14 for the CMAS-BW pilot plant.

3. Complete Mixing Activated Sludge - Continuous Solids Wasting (CMAS-CW) vs Complete Mixing Activated Sludge - Batch Solids Wasting (CMAS-BW)

These two systems, in which the feeding schedule was the same, but the solids wasting procedure was different, were operated on a potassium acetate substrate at a volumetric loading of 120 lbs COD/1000 cu ft/d and an SRT of 215 days. The higher volumetric loading was used to produce a satisfactory Mv concentration at a somewhat shorter SRT. Both pilot plants were continuously-fed, in one, 40 per cent of the mixed liquor was withdrawn once a day and replaced with Cambridge, Mass., tap water. In the other, about 50 ml of mixed liquor was discharged about every hour (total 40 per cent of the aeration volume in one day) through a dosing syphon. The results of this study are shown in Figure 3. While these units did not operate as well as was hoped, and the mixed liquor volatile solids concentrations were quite variable, they did operate nearly the same. The method of wasting solids did not affect the level of the mixed liquor volatile solids concentrations.

4. Summary and Discussion

A comparison of Figures 1, 2 and 3 shows that the method of feeding a substrate has a strong influence on the resulting level of mixed liquor volatile solids while the method of wasting solids does not. Batch feeding, which produces a substrate unlimited situation at each feeding similar to that which occurs at the head end of a conventional activated sludge plant, causes higher resultant solids than continuous feeding, which maintains a continual low substrate concentration,

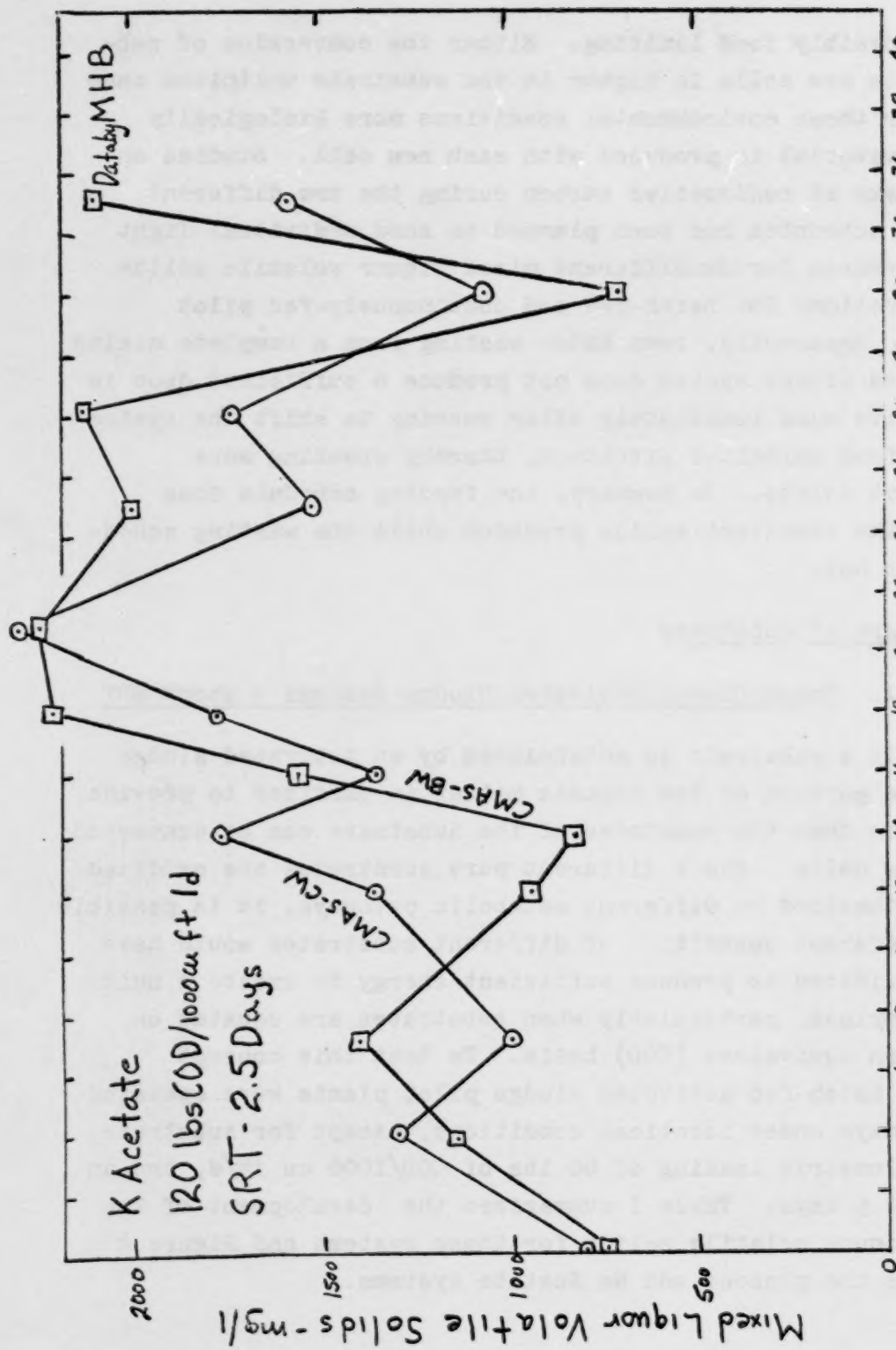

Figure 3 Comparsion of CMAS-BW and CMAS-CW

quite possibly food limiting. Either the conversion of substrate to new cells is higher in the substrate unlimited case or under these environmental conditions more biologically "hard" material is produced with each new cell. Studies on the uptake of radioactive carbon during the two different feeding schedules had been planned to shed additional light on the reason for the different mixed liquor volatile solids concentrations for batch-fed and continuously-fed pilot plants. Apparently, even batch wasting from a complete mixing activated sludge system does not produce a sufficient drop in the active mass immediately after wasting to shift the system into a food unlimited situation, thereby creating more resultant solids. In summary, the feeding schedule does affect the resultant solids produced while the wasting schedule does not.

B. Nature of Substrate

1. Conventional Activated Sludge Systems - Short SRT

As a substrate is metabolized by an activated sludge system a portion of the organic matter is oxidized to provide energy so that the remainder of the substrate can be converted into new cells. Since different pure substrates are oxidized and synthesized by different metabolic pathways, it is possible that different quantities of different substrates would have to be oxidized to produce sufficient energy to create a unit of protoplasm, particularly when substrates are equated on an oxygen equivalent (COD) basis. To test this concept several batch-fed activated sludge pilot plants were operated for 19 days under identical conditions, except for substrate, at a volumetric loading of 60 lbs of COD/1000 cu ft/d, and an SRT of 2.5 days. Table I summarizes the development of the mixed liquor volatile solids for these systems and Figure 4 compares the glucose and Na Acetate systems.

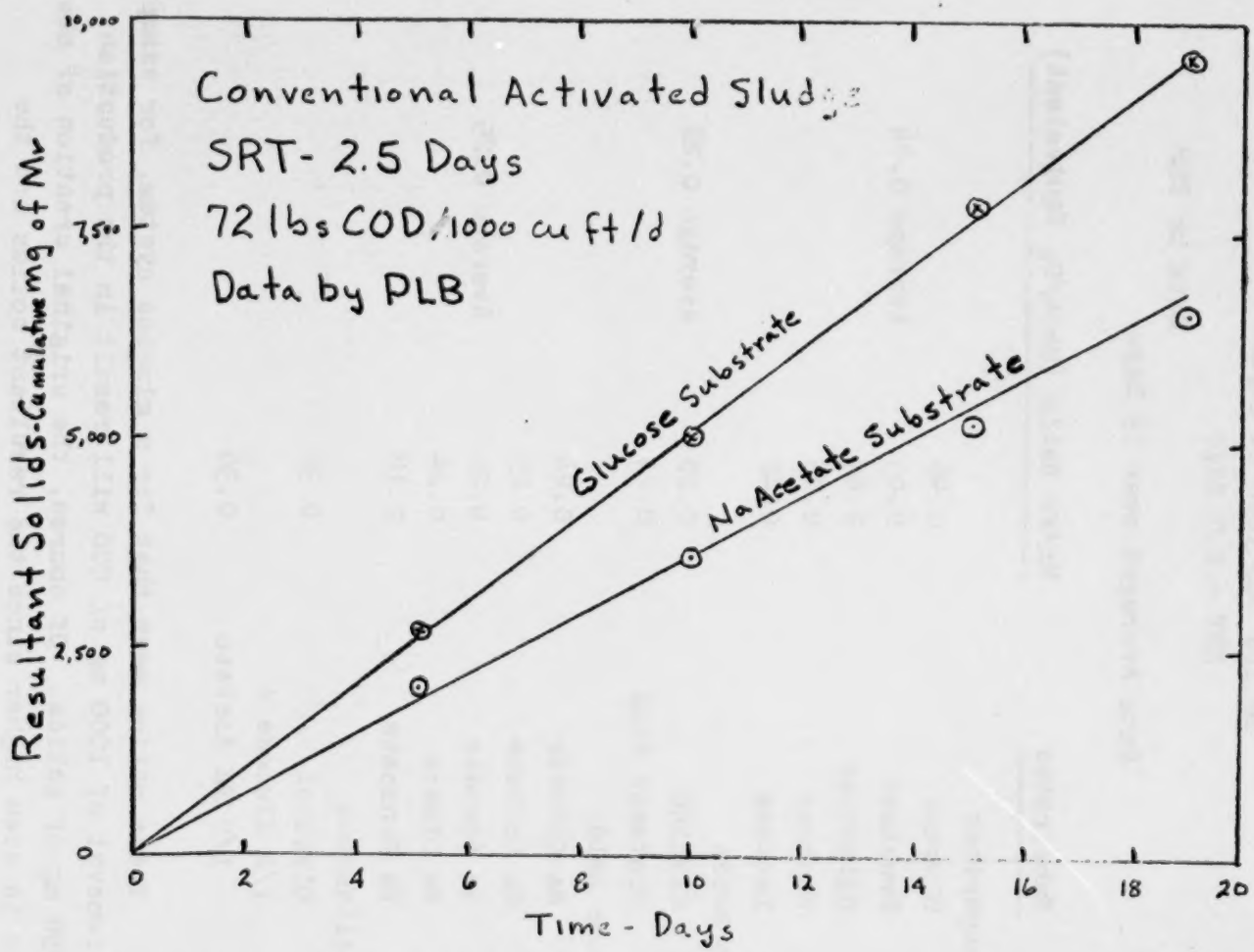

Figure 4 Comparison of Glucose and Na Acetate Substrates

TABLE I

COMPARISON OF Mr/Fr RATIOS FOR VARIOUS SUBSTRATES

Conventional Activated Sludge (Batch-Fed)
60 lbs COD/1000 cu ft/d
SRT - 2.5 days

Data by PLB

Data Averaged over 19 Days

Substrates	Mr/Fr Ratio (Mass/O_2 Equivalent)	
Carbohydrates		
Glucose	0.46	
Fructose	0.43	Average 0.44
Galactose	0.44	
Sucrose	0.46	
Lactose	0.40	
Amino Acids		
Glycine	0.20	Average 0.22
Glutamic Acid	0.24	
Organic Acids		
Na Citrate	0.24	
Na Lactate	0.25	
Na Acetate	0.29	Average 0.25
Na Oleate	0.24	
Na Benzoate	0.18	
Miscellaneous		
Glycerol	0.36	
1/2 Glucose + 1/2 Na Acetate	0.36	

These ratios mean that for a glucose system, for example, the removal of 1000 mg of COD will result in the production of 450 mg of solids. Of course, the original creation of new cells is even higher since the resultant solids are the

difference between the original creation of new cells minus the destruction of solids by endogenous metabolism. The relative values indicate that, at least under these conditions, systems metabolizing carbohydrates would produce about 80 per cent more volatile solids as systems metabolizing organic acids or amino acids, all other variables being equal. On the basis of an equal quantity of oxidation, however, carbohydrates produce about 2.4 times the weight of solids than amino acids, on the average.

2. Complete Mixing Activated Sludge

a. Short Solids Retention Time

Several continuously-fed pilot plants were operated on various substrates at SRT's between three and ten days. The volumetric loading on these systems was either 50 or 60 lbs COD/1000 cu ft/d. The resultant solids ratio was calculated from the average value of the mixed liquor volatile solids concentration and the per cent solids wasting. These data are summarized in Table II.

TABLE II

COMPARISON OF Mr/Fr RATIOS FOR VARIOUS SUBSTRATES
Complete Mixing Activated Sludge (Continuously-Fed)

Substrate	Volumetric Loading lbs COD/1000 cu ft/d	SRT days	Mr/Fr Ratio Mass/O_2 Equiv.	Data by
Na Acetate	60	3	0.15	PLB
" "	"	4	0.16	"
0.6 Nut br. + 0.2 Na Ol + 0.2 Starch	50	3.7	0.14	MWT
Na Acetate	60	10	0.13	MHB
Na Oleate	60	10	0.13	"
Glucose	"	"	0.18	"
Starch	"	"	0.18	"

b. Long Solids Retention Time

In this study three continuously-fed pilot plants were operated at a volumetric loading of 30 lbs COD/1000 cu ft/d and an SRT of 200 days. Each unit was fed a different substrate using a 2500 ml pilot plant and removing a 25 ml sample for mixed liquor volatile solids every other day. Most of the solids were retained by a small settling tank, the rest were recycled by centrifugation. Each system was Waring Blended every other day. The units were wasted, but not sampled for the first 15 days of operation for the Na Acetate and glucose units and 21 days for the glycine system. The entire test lasted 41 days. The results are shown in Table III.

TABLE III

COMPARISON OF Mr/Fr RATIOS FOR VARIOUS SUBSTRATES

Complete Mixing Activated Sludge (Continuously-Fed)

30 lbs COD/1000 cu ft/d

SRT - 200 days

Data Averaged from Day 15-41 Data by MWT

Substrate	Mr/Fr Ratio (Mass/O_2 Equivalent)
Glucose	0.08
Glycine	0.07
Na Acetate	0.06
0.6 Nut. Br. + 0.2 Na Ol + 0.2 Starch	0.07

3. Summary and Discussion

In a conventional activated sludge system operated at a fairly short solids retention time, the substrate metabolized has a strong influence on the resultant solids ratio, Mr/Fr.

Some data taken by Washington and reported by Symons (2) indicates that this influence lessens as the SRT increases. These data are summarized in Table IV. The data from the

TABLE IV

COMPARISON OF Mr/Fr RATIOS FOR VARIOUS SUBSTRATES (2)

Conventional Activated Sludge (Batch-Fed)
40 lbs COD/1000 cu ft/d

Substrate	SRT Days	Mr/Fr Ratio Mass/O_2 Equivalent
Na Acetate	150	0.16
Glucose	about 100	0.12 (Average of 2)
Glycine	about 100	0.07 (Average of 2)

glycine system may be misleading as those activated sludge systems did not operate in a normal manner. Discounting this, the ratios were quite close when solids wasting was small. This indicates that the higher resultant solids arising from the metabolism of carbohydrates at low SRT's is caused by the formation of more new cells and not due to the formation of cells rich in biologically "hard" materials. If the latter were true, when the Mr/Fr ratio became a minimum in high SRT systems, it would have been higher in the glucose system due to this "hard" material. The similarity of the data in Table IV indicates that the "remains" of microorganisms metabolizing different substrates are about the same.

Similar conclusions may be drawn for complete mixing activated sludge, as a comparison of Table II and III shows. One additional feature is evident in Table II, however. These data would seem to indicate that even at low SRT's substrate does not have the strong influence on the Mr/Fr ratio in the food limited case as in the food unlimited

situation reported in Table I. These data are limited, as are those in Table IV, however, and further work is indicated in these areas to yield final conclusions.

The result on the mixture of Na Acetate and glucose reported in Table I indicate that the results of individual substrates are additive. Some inconsistency arises when comparing these data with that taken on other mixtures, however. A conventional activated sludge system loaded at 52 lbs COD/1000 cu ft/d, operated at an SRT of 2.5 days, and metabolizing a 60 per cent nutrient broth, 20 per cent carbohydrate, 20 per cent organic acid substrate (percentages on a COD basis) yielded a Mr/Fr ratio (mass/O_2 equivalent) of 0.41. Calculations based on the data in Table I indicate a ratio of 0.26. This discrepancy is unresolved at the present time. However, even if the absolute values in Table I are incorrect, their relative values are still important. The systems were all operated in an identical manner, but produced different quantities of solids as the substrate varied.

C. Varying Volumetric Loading

1. Conventional Activated Sludge

One brief study was performed on conventional activated sludge systems to show the influence of volumetric loading. The data are summarized in Table V. From this limited data, volumetric loading would seem to influence the resulting solids produced very little.

TABLE V

COMPARISON OF Mr/Fr RATIOS FOR TWO VOLUMETRIC LOADINGS

Conventional Activated Sludge

Data by PLB

Substrate	SRT Days	Volumetric Loading lbs COD/1000 cu ft/d	Mr/Fr Ratio Mass/O_2 Equivalent
Glucose	2.5	30	0.44
Glucose	2.5	60	0.46
Na Acetate	2.5	30	0.30
Na Acetate	2.5	60	0.29

2. Complete Mixing Activated Sludge

a. Long Solids Retention Time

One study was made to determine the influence of volumetric loading on the Mr/Fr ratio in the complete mixing activated sludge system operating at a long solids retention time. The results of this experiment are given in Table IV. Some influence is seen here, especially in the lower loading.

TABLE VI

COMPARISON OF Mr/Fr RATIOS FOR THREE VOLUMETRIC LOADINGS

Complete Mixing Activated Sludge

0.6 Nutrient Broth + 0.2 Na Oleate + 0.2 Starch Substrate

SRT = 200 days

Data by MWT

Volumetric Loading lbs COD/1000 cu ft/d	Mr/Fr Ratio Mass/O_2 Equivalent
15	0.04
30	0.07
60	0.07

b. Short Solids Retention Time

Ten complete mixing activated sludge systems were operated at a solids retention time of 0.25 days on a mixed soluble substrate at volumetric loadings from 60 to 1700 lbs COD/1000 cu ft/d. The purpose of this study was two-fold; one, to show the relationship, if any, between the Mr/Fr ratio and volumetric loading; and, two, to explore the possibility of operating activated sludge units at these high volumetric loadings. This study is reported in detail in a thesis for the degree of Sanitary Engineer at the Massachusetts Institute of Technology by Raymond H. Johnson, Jr., June 1963 (15), and will be summarized here. Figure 5 shows the Mr/Fr ratios for the ten systems, the spread indicates the standard deviation of each point. While the data is scattered, it seems clear that the Mr/Fr ratio is not dependent on the volumetric loading.

Figure 6 shows the operation of these same ten systems with respect to the removal and conversion of COD. The COD fed to these systems was all soluble. As it was metabolized, some was converted to carbon dioxide and water, some was converted to new solids, and some was untouched. Although the data was somewhat scattered, only about 11 per cent of the feed was untouched, but of the 89 per cent which was biologically manipulated, about 43 per cent was lost (38 per cent of the food added), the remainder was converted to new cells. The problem in operating systems in this manner therefore is the development of a method for clarifying the effluent to obtain 89 per cent removal and destroying the great quantities of solids which are created. This problem will be discussed in the last section of this report. Systems operated in this manner have a potential of removing great quantities of COD rapidly, in small aeration basins.

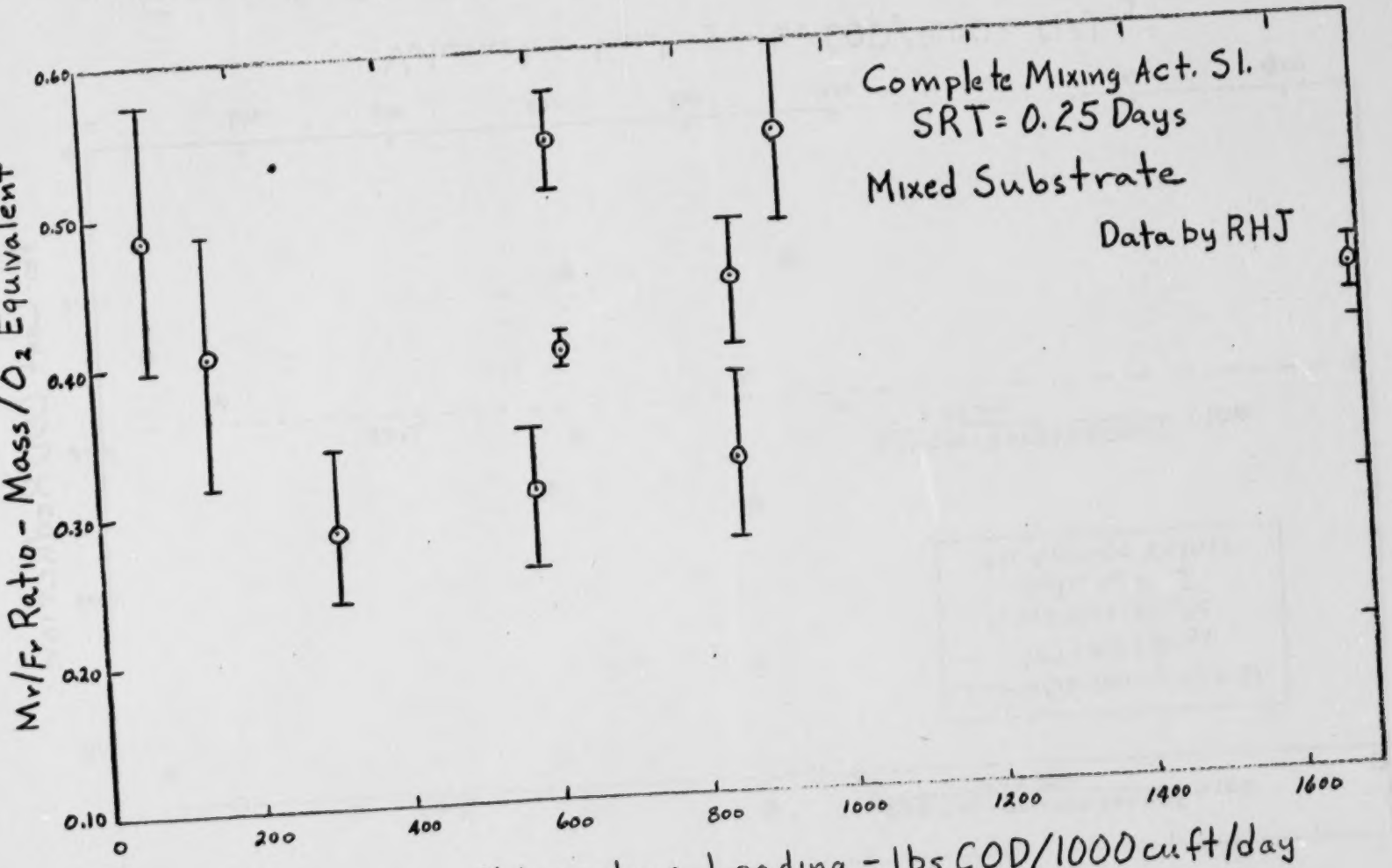

Figure 5 Influence of Volumetric Loading on Solids Production

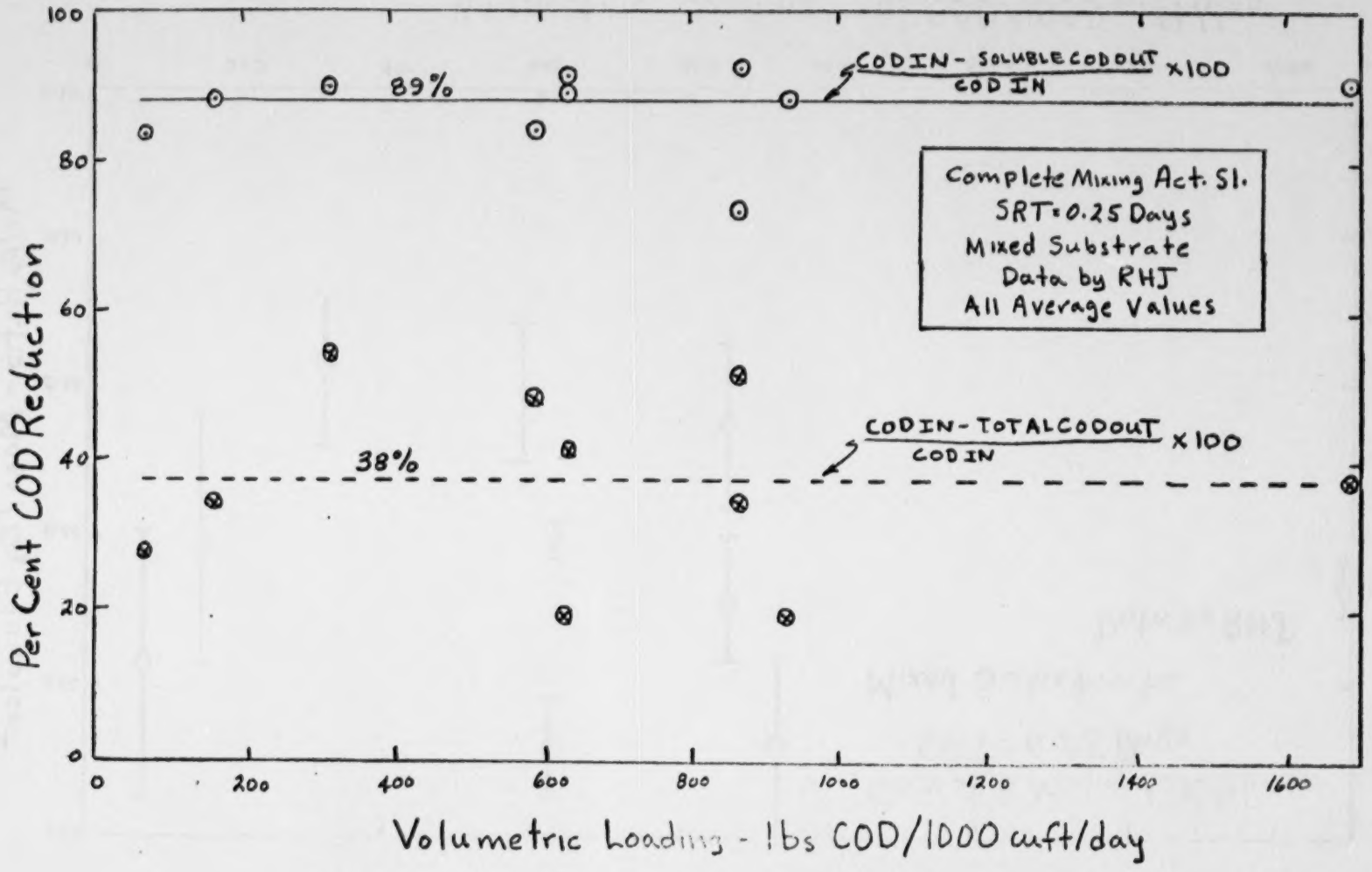

Figure 6 Operation of Highly Loaded Complete Mixing Activated Sludge

3. Summary and Discussion

The studies here indicate that in general the volumetric loading on an activated sludge system, either conventional or or complete mixing, has little if any influence on the Mr/Fr ratio. Some influence was noted for the one complete mixing system operated at a very low volumetric loading, however.

D. Varying Solids Retention Time

1. Conventional Activated Sludge

In order to determine the effect of variations in solids wasting in the conventional activated sludge process, all other conditions being equal, ten batch-fed activated sludge pilot plants were operated on a synthetic sewage mixture consisting of sodium lactate, glucose, and nutrient broth. The volumetric loading was 52 lbs of COD/1000 cu ft/d and the SRT's were varied from 1.3 to 60 days (1.7 to 80 per cent wasting per day). These systems were operated for 42 days and the mixed liquor volatile solids concentrations in all but the three longest SRT systems were quite steady over the last 10 days. All systems maintained very good (92 per cent) removal of COD. The results with respect to solids production are shown in Figure 7.

These data show an expected pattern of increasing production of resultant solids with increasing wasting, that is, shorter solids retention time. The maximum Mr/Fr ratio was 0.42 while the minimum was 0.17. The data for the 30 and 60 day systems might be somewhat high as the systems were only operated for 42 days. Data previously taken by Symons (1) showed a similar pattern. The ratios are somewhat lower for the Na Acetate substrate than for the mixed substrate, which is in agreement with Table I. The presence of glucose in the mixture was the probable cause of the higher ratios. A

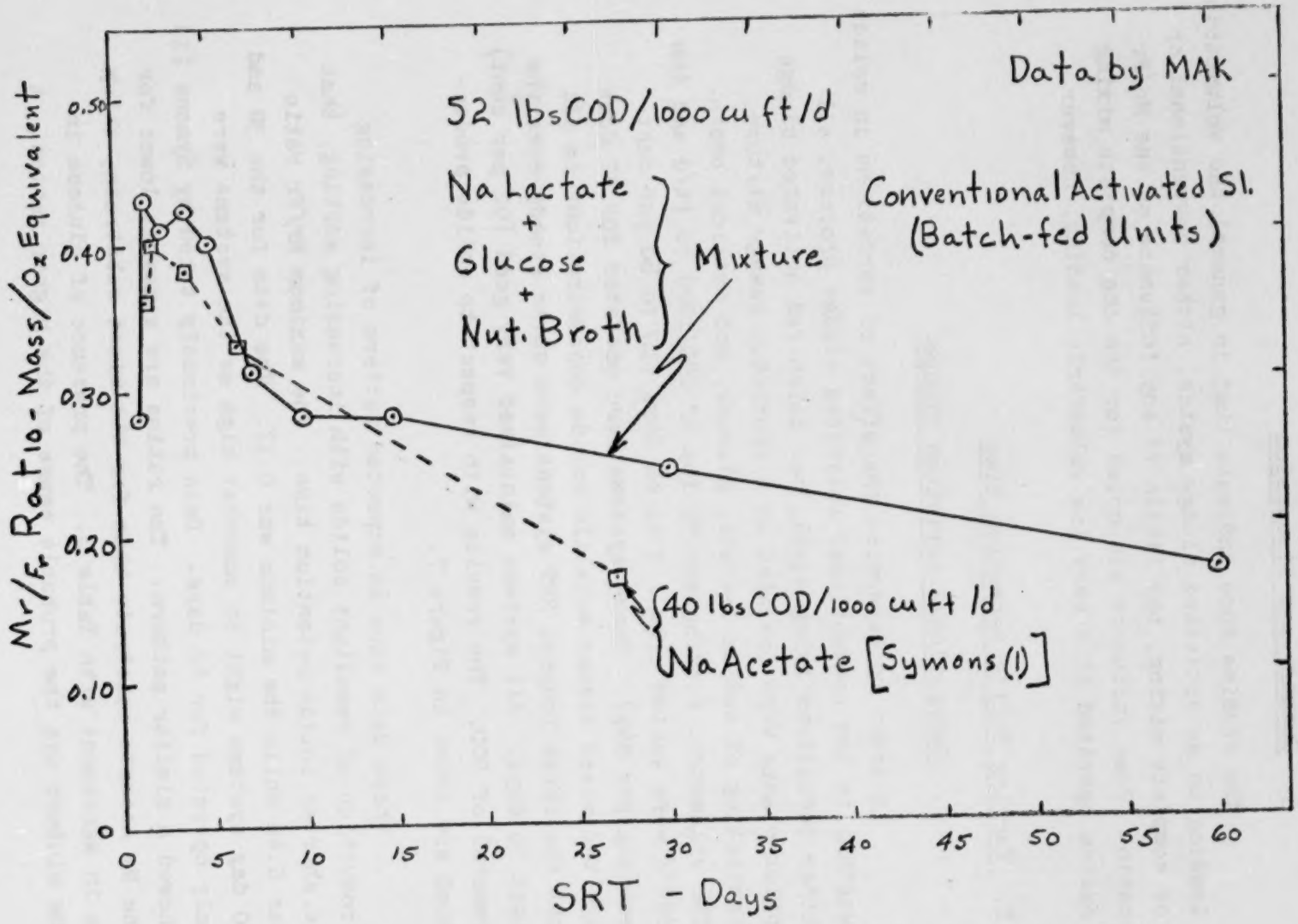

Figure 7 Variation of Resultant Solids with SRT

comparison of the ratio at an SRT of 2.5 days from Symons (1) - 0.39 - with that ratio given in Table I for Na Acetate of 0.29 indicates some disagreement. This is unresolved at this time. The volumetric loadings were different in the two systems, however this should only have a slight effect.

One surprising feature of this study was the decline in the Mr/Fr ratio as the SRT shortened from 1.5 to 1.3 days for the mixed substrate and from 2.1 to 1.5 days for the Na Acetate substrate. A similar effect, but not as pronounced, was noticed in another set of experiments. These data are given below. The changes which occur in very heavily wasted conventional activated sludge systems which cause a decrease in resultant solids is unknown at this time. Possibly a change in predominant organism occurs under these conditions. Only further research will reveal the answer.

TABLE VII

COMPARISON OF Mr/Fr RATIOS FOR VARIOUS SRT'S

Conventional Activated Sludge (Batch-Fed)

60 lbs COD/1000 cu ft/d

Data Averaged Over 19 Days

Data by PLB

Substrate	SRT - Days	Mr/Fr Ratio - Mass/O_2 Equivalent
Glucose	2.5	0.46
Glucose	1.5	0.40
Na Acetate	2.5	0.29
Na Acetate	1.5	0.25

2. Complete Mixing Activated Sludge Systems

In order to determine the influence of variations in solids wasting on the resultant solids in the complete mixing activated sludge system, six units were operated on a Na

Acetate substrate at a volumetric loading of 60 lbs COD/ 1000 cu ft/d with SRT's varying from 1 to 6 days. Since this study was performed before it was determined that the wasting schedule had little influence on the production of resultant solids, these systems were operated by the more difficult, less reliable method of continuous wasting.

This was attempted by having a pilot plant without any settling section and discharging mixed liquor volumes equal to the liquid volume of feed. In an attempt to discharge a representative sample, the mixed liquor discharged periodically in 50 to 100 ml slugs through a dosing syphon. This arrangement was not entirely satisfactory, however, as the mixed liquor volatile solids concentrations varied widely from sampling to sampling. It was not determined whether or not this was an important biological action worth investigating further, or an artifact caused by the mechanics of the system. In either case the operation was not very satisfactory.

In spite of these difficulties, the average values of the mixed liquor volatile solids concentrations over the 60 days of operation did give some insight into the variation of the resultant solids with varying SRT. These data are plotted in Figure 8. These data show two features, one the range of SRT's studied did not cover a wide enough range to show much variation in the Mr/Fr ratio and two, a comparison of these data with some data from Figure 7 again shows the influence of loading schedule on the resultant solids. Also the uniformity of the Mr/Fr ratio over a similar SRT range for conventional activated sludge is evident in Figure 8. (1)

3. Summary and Discussion

In an effort to show the dependancy of the Mr/Fr ratio on the solids retention time or sludge wasting, data from many different sources has been summarized in Tables

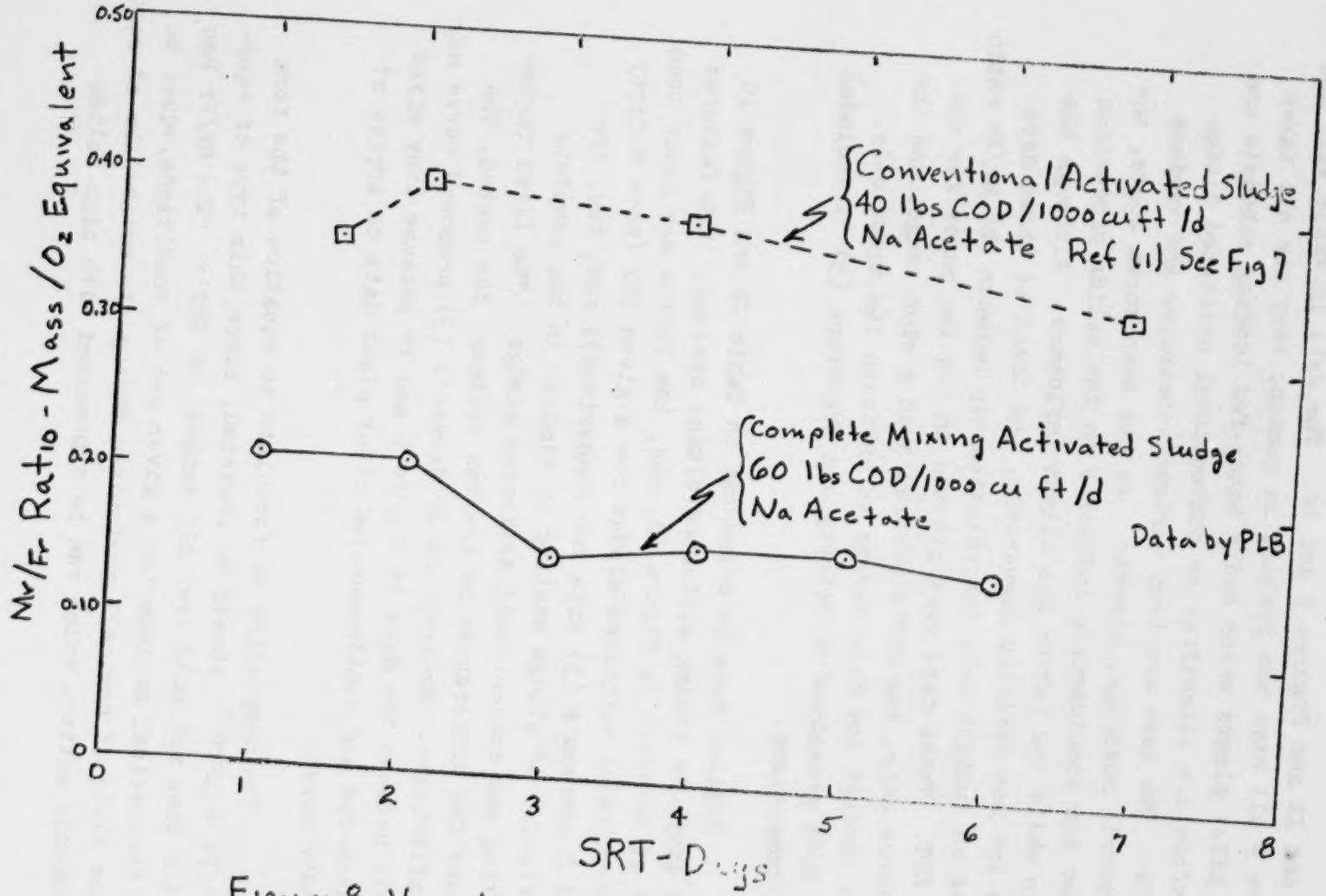

Figure 8 Variation of Resultant Solids with SRT

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VIII and IX and Figures 9 and 10. The data in Table VIII and Figure 9 all have one feature in common; they are all taken from pilot plants which had a batch-fed loading schedule and therefore are classified as conventional activated sludge plants. The data are from various substrates and various volumetric loadings, however. As has been shown above, the former has considerable influence on the solids production ratio while the latter has slight influence. Although the data are not strictly comparable, the trend of these data gives an insight into the relationship between the Mr/Fr ratio and SRT. These data were plotted on log-log paper for convenience only, the SRT's covered such a wide range, and the line through the data serves to indicate the trend only. The data presented by McCarty and Broderson (3) is included for comparison.

Similar data is presented in Table IX and Figure 10 for complete mixing activated sludge systems. Two features are of interest in Figure 10; one, the ratios are lower than conventional activated sludge for a given SRT (note McCarty and Broderson's (3) data for comparison); and, two, the influence of sludge wasting is similar in the complete mixing and conventional activated sludge. The first factor shows the differences in the two systems; the second, the similarities. McCarty and Broderson's (3) proposed curve may fall between the data in Figure 9 and 10 because they mixed batch-fed and continuous-fed pilot plant data to arrive at their curve.

The temptation to formulate an equation of the form $Mr/Fr = mSRT^{-x}$ should be resisted, since this type of equation does not hold over all ranges of SRT's. The Mr/Fr has a theoretical maximum for a given set of conditions, equal to the yield of new cell protoplasm during the metabolism of some organic matter, which can be approached with high solids

TABLE VIII

SUMMARY OF Mr/Fr RATIO DATA
FOR
CONVENTIONAL ACTIVATED SLUDGE

Substrate	Volumetric Loading lbs COD/1000 cu ft/d	SRT Days	Mr/Fr Ratio Mass/O_2 Equiv.	Data by
Mixed -	52	1.3	0.28	MAK
(Na Lactate +	"	1.5	0.43	
Starch +	"	2.5	0.41	
Nut. Broth)	"	3.8	0.42	
"	"	5.0	0.40	
"	"	7.5	0.31	
"	"	10	0.28	
"	"	15	0.28	
"	"	30	0.24	
"	"	60	0.17	
Na Acetate	40	1.5	0.36	Symons (1)
"	"	2.1	0.40	
"	"	3.9	0.38	
"	"	6.8	0.33	
"	"	27	0.17	
Na Acetate	40	150	0.16	Washington (2)
Mixed-(NaOl+ Starch + Nut.Br.)	50	3.7	0.35	MWT
Mixed-(Glucose+ Na Ac)	60	2.5	0.36	PLB

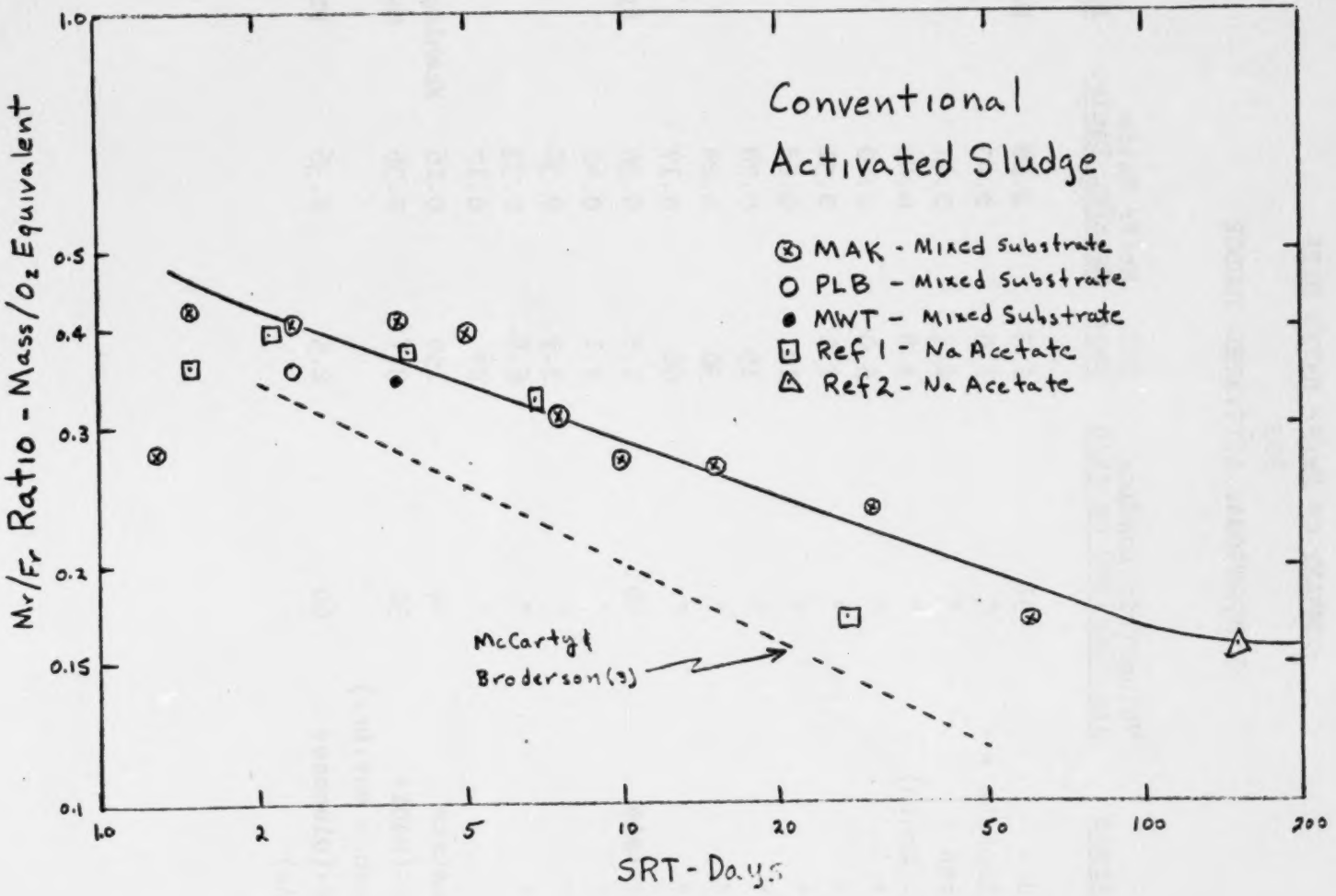


Figure 9 Summary of Data for Conventional Act. Sludge

TABLE IX

SUMMARY OF Mr/Fr RATIO DATA

FOR

COMPLETE MIXING ACTIVATED SLUDGE

Substrate	Volumetric Loading lbs COD/1000 cu ft/d	SRT Days	Mr/Fr Ratio Mass/O_2 Equiv.	Data by
Na Acetate	60	1	0.21	PLB
"	"	2	0.21	
"	"	3	0.15	
"	"	4	0.16	
"	"	5	0.16	
"	"	6	0.15	
Na Acetate	40	156	0.11	Washington (2)
Mixed-(NaOl+ Starch+Nut.Br.)	60	200	0.07	MWT
Mixed-(NaAc.+ Sucrose+Nut.Br.)	60	0.25	0.48	(Ave. RHJ of several)
Glucose	30	200	0.08	MWT
Glycine	30	200	0.07	"
Na Acetate	30	200	0.06	"
Mixed-(NaOl+ Starch+Nut.Br.)	50	3.7	0.14	"
Na Acetate	60	10	0.13	MHB
Na Oleate	60	10	0.13	MHB
Glucose	60	10	0.18	"
Starch	60	10	0.18	"

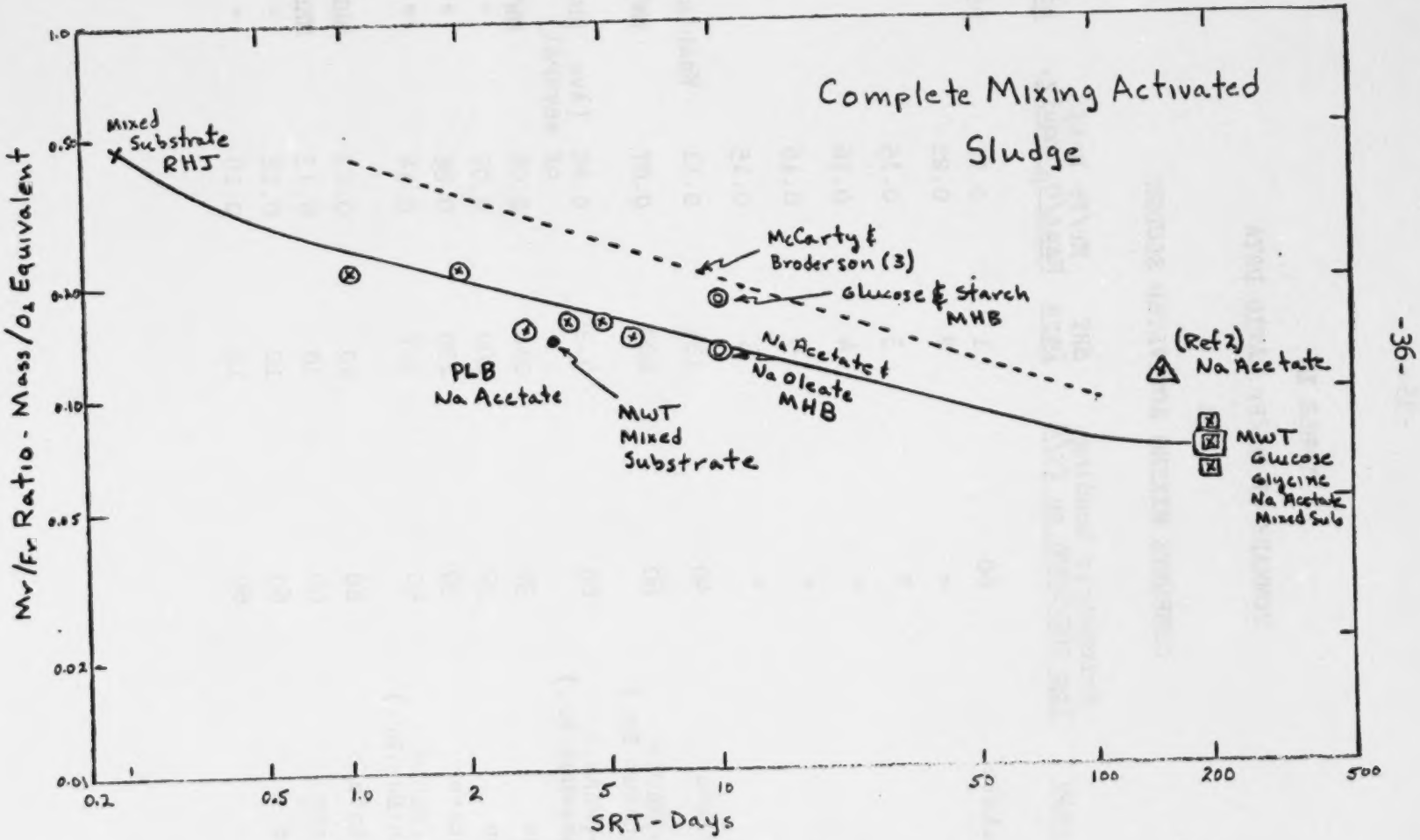


Figure 10 Summary of Data for Complete Mixing Act. Sludge

wasting, low SRT. Also, Mr/Fr has a theoretical minimum for a given set of conditions, equal to the inert biological mass produced per day, which can be approached with little solids wasting, high SRT. Radioactive carbon studies had been planned to evaluate the maximum. An estimate of these data for mixed soluble substrates with a volumetric loading near 60 lbs COD/1000 cu ft/d is given in Table X.

TABLE X

New Cell Yield/Unit COD Removed and Inert Mass Produced/ Unit COD Removed

System	New Cell Yield Mass/O_2 Equivalent	Inert Mass Produced Mass/O_2 Equivalent
Conventional Activated Sludge	0.60 - 0.70	0.16
Complete Mixing Activated Sludge	0.50*	0.09
McCarty and Broderson (3)	0.43	0.08

*Figure 5 shows some lower values. This was based on the system volumetrically loaded at 60 lbs COD/1000 cu ft/d.

From these data an estimate of the rate of solids destruction under plant operating conditions may be made, using the form of the formula presented by McCarty and Broderson (3). Table XI summarizes these data. The reason for the very high rate determined for complete mixing activated sludge is not known at this time, possibly due to a high value for the new all yield.

TABLE XI

SUMMARY OF RATES OF SOLIDS DESTRUCTION UNDER FED CONDITIONS

Sample	Rate of Solids Destruction	Reference
Complete Mixing Activated Sludge	0.58 per day	Table X Figure 10
Conventional Activated Sludge	0.21 to 0.29 per day	Table X Figure 9
Activated Sludge	0.18 per day	(3)

E. Varying Nitrogen Supplementation - Complete Mixing Activated Sludge

1. Influence on Solids Production and Behavior of System

Sawyer and his co-workders (4) established a BOD/N ratio of 20/1 to permit the full unrestriced growth of conventional activated sludge, although they admitted that higher ratios were possible. They placed a suggested lower limit of 7 per cent nitrogen in the volatile mass to obtain a good settling sludge. Symons (1) confirmed these latter concepts, pointing out that COD/N ratios as high as 85/1 were acceptable provided that the SRT was maintained as high as possible. Also he suggested that the lower nitrogen content in the volatile mass was due to the formation of nitrogen-free inert biological mass. Finally, he predicted increased resultant solids under nitrogen starved conditions, due to the increased production of biologically inert mass.

Washington, reported by Symons (2), working with richly supplemented, long SRT, complete mixing activated sludge, found that the inert biological mass contained some nitrogen and, therefore, in spite of the creation of new mass and the

destruction of old occurring simultaneously, all of the nitrogen could not be recycled. There was a continuing nitrogen demand of 100/1 to 150/1 in terms of COD/N ratio.

To study the influence of nitrogen deficiency on the behavior of complete mixing activated sludge operating in the moderate solids aeration regime, two experiments were performed. These tests were made in pilot plants without settling chambers, as have been previously described. Table XII summarizes the conditions of the experiment.

TABLE XII

SUMMARY OF NITROGEN DEFICIENCY EXPERIMENTS

Substrate	Vol. Loading lbs COD/ 1000 cu ft/d	COD/N Ratios	SRT Days	Length of Test Weeks
K Acetate	55	33/1, 39/1, 46/1, 57/1, 57/1, 77/1, 115/1	about 7	20
K Lactate	60	57/1, 77/1 115/1, 230/1 310/1, 460/1	about 7	12

The pertinent data, averaged over the entire test period, is presented in Figure 11 to 13. These data are plotted on semi-log paper for convenience only. In the interpretation of these data two factors are important; one, since no solids settling was employed, the bulkiness of some of the sludges did not upset the system. As a general rule, a nitrogen content of the mixed liquor volatile solids below 4 to 5 per cent caused a bulky sludge. Two, nitrogen fixation began spontaneously in the three highest COD/N ratio systems on the K Lactate substrate and this may have caused these data to be somewhat atypical. They were included in all of the Figures,

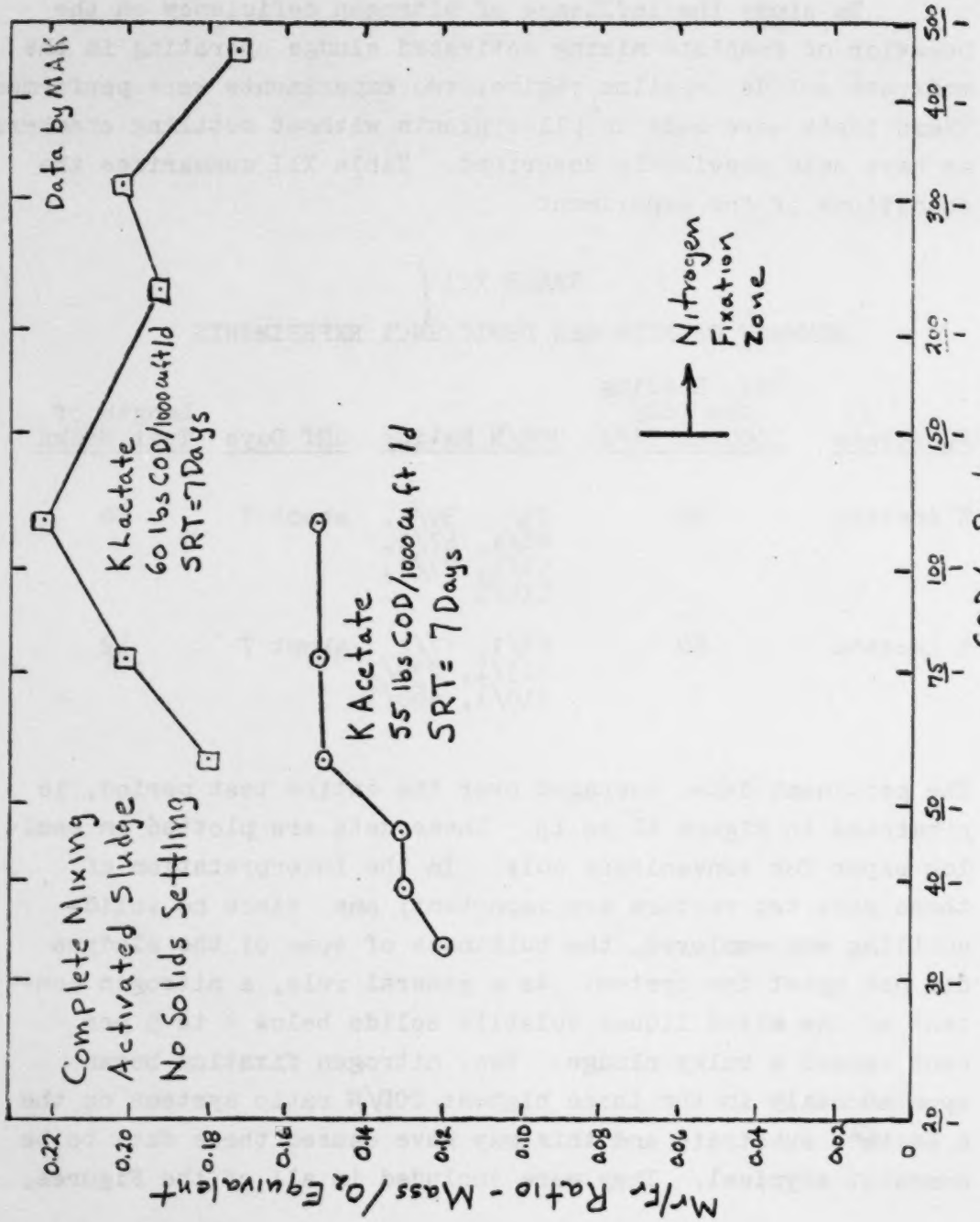


Figure 11 Influence of Nitrogen Fed on Solids Production

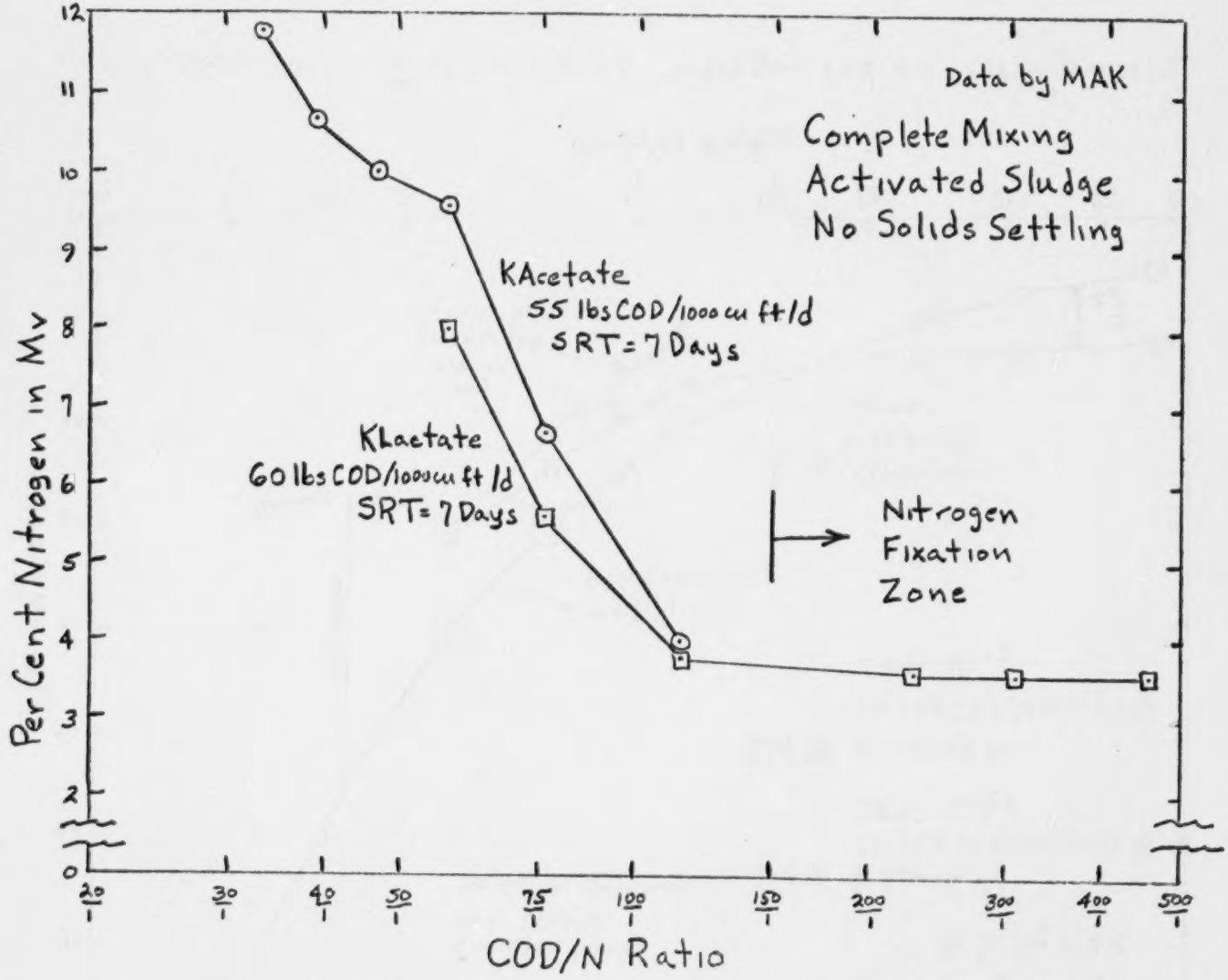


Figure 12 Influence of Nitrogen Fed on % N in Mv

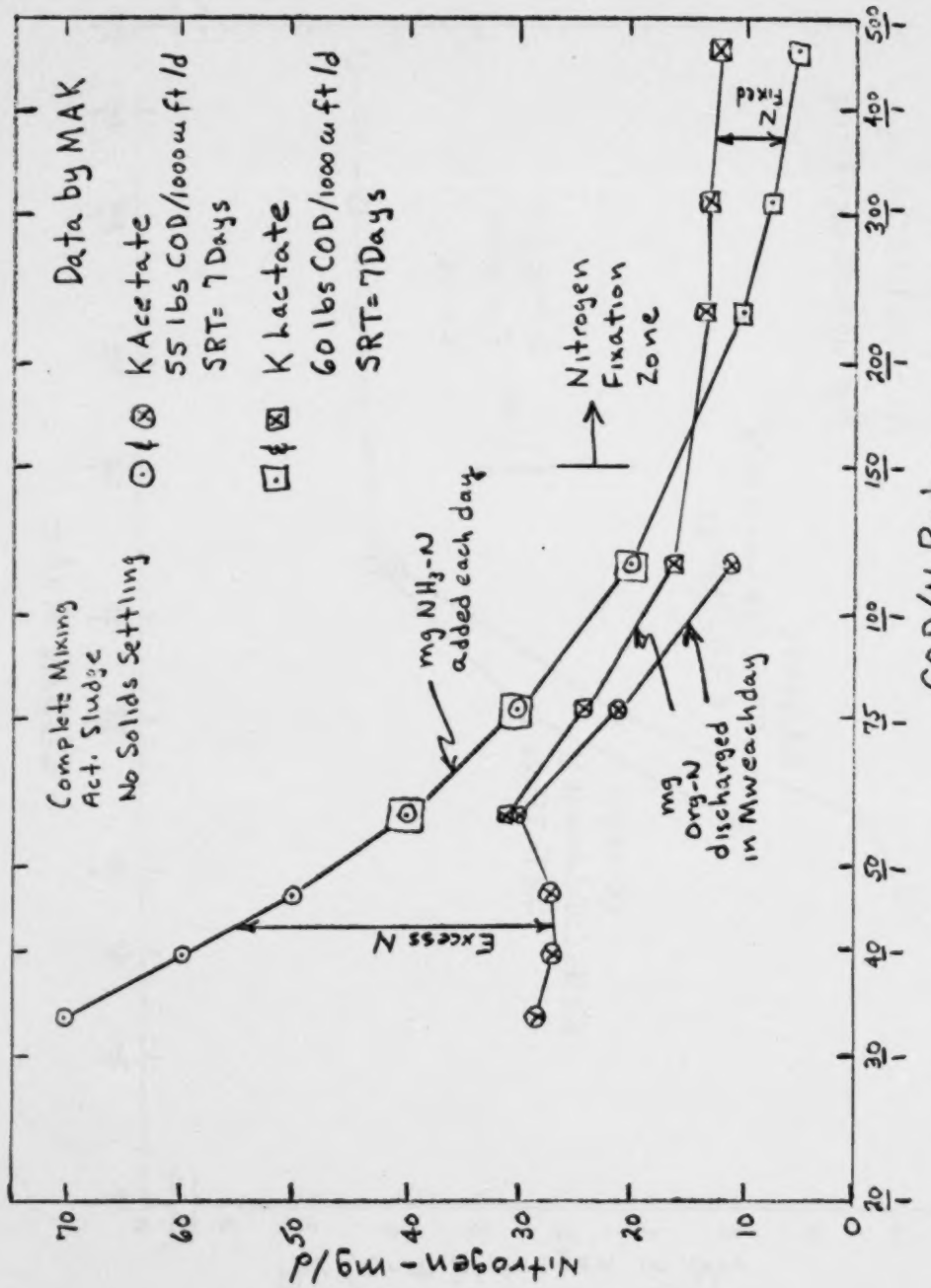


Figure 13 Influence of Nitrogen Fed on Nitrogen Use

however. A complete discussion of nitrogen fixation appears at the end of this section.

Figure 11 shows an increase in the Mr/Fr ratio as the quantity of nitrogen fed to the system is decreased, ignoring the nitrogen fixation data. This agrees with the data obtained by Symons (1) on conventional activated sludge. Although the planned radioactive carbon study to demonstrate this was not performed, the increase in resultant solids production is probably due to the formation of more biologically "hard" material which is more slowly destroyed rather than being due to more new cells being formed. Since the per cent nitrogen in the solids declines with increasing COD/N ratio, Figure 12, this biologically "hard" material is quite likely low in organic nitrogen.

This increase in Mr/Fr ratio when the COD/N rises was noticed in many other related experiments, including one in which an entirely closed system was operated on pure oxygen and the CO_2 produced per day was measured by daily gas analysis. When the COD/N ratio was changed from 25/1 to 1000/1, the CO_2 produced per day promptly declined, indicating more resultant solids production. Some future research should be done to determine the exact cause of this resultant solids increase.

Figures 12 and 13 show how a complete mixing activated sludge system reacts to nitrogen deficiency. When the nitrogen is plentiful, COD/N ratios of 33/1 to 57/1, the decrease in nitrogen added each day has little influence on the system - only a slight decline in per cent nitrogen in the mixed liquor volatile solids. As the quantity of nitrogen lost in the wasted solids each day approached 75 per cent of the nitrogen supplied each day, however, the system reacts to prevent such a high loss in nitrogen. This is accomplished by the development of a nitrogen-lean activated sludge. For the COD/N ratios of 57/1, 77/1, and 115/1 the system compensated for the declining nitrogen addition by producing resulting solids

lower in nitrogen content, therefore, the nitrogen discharged each day was never greater than 80 per cent of the nitrogen added each day.

At a COD/N ratio of 115/1 the mixed liquor solids contained about 4 per cent nitrogen. Attempts to lower this percentage still further by increasing the COD/N ratio were not successful as the systems began to fix nitrogen. This implies that 4 per cent nitrogen in the mixed liquor volatile solids is about as low as a system will operate. Nitrogen contents below this value are somewhat academic anyway, as very bulky sludges often develop under these conditions.

The nitrogen requirements of a complete mixing activated sludge system is related to the Mr/Fr ratio which is related to the solids wasting, and to the per cent nitrogen in the solids. If 5 per cent nitrogen could be tolerated in the mixed liquor solids, an increase in Mr/Fr ratio of about 25 per cent would be noticed based on Figure 11. Further, if 80 per cent of the nitrogen added could be discharged, the required COD/N ratio could be calculated by:

$$\frac{COD}{N} = \frac{Fr/Eff.}{\frac{1.25 \times Mr/Fr \times 0.05 \times Fr}{0.8}} = \frac{12.8}{Mr/Fr \times Eff.}$$

Assuming an efficiency of COD removal of 92 per cent, and based on the SRT vs. Mr/Fr curve in Figure 10, at low SRT's a COD/N ratio of about 28 /1 is required while at long SRT's about 176/1 would be acceptable. This is higher than determined by Washington, reported by Symons (2), but his were maximum, not minimum, requirements.

This concept is extrapolated from two experiments. Further research is needed to determine if systems could be successfully operated over wide ranges of solids wasting with a nitrogen content of 5 per cent in the mixed liquor volatile solids. Also, all of these tests were performed with ammonia

nitrogen as the nitrogen supplement, possibly the use of nitrite or nitrate nitrogen would alter the situation somewhat.

2. Influence on Efficiency

Under conditions of nitrogen deficiency a system compensates by creating an activated sludge of lower nitrogen content than normal. This sludge is not as efficient with respect to metabolism of organic matter as normal sludge, however. As seen in Figure 14, as the COD/N ratio increases, the per cent COD removal decreases. Since the removal of organic matter is the goal of any waste treatment device, this loss in efficiency with decreasing nitrogen content in the mixed liquor volatile solids was thoroughly evaluated. This evaluation will form the major portion of a doctoral thesis at the Massachusetts Institute of Technology, by Mohamed H. Bechir (5), tentative 1963, but the sections of the research supported in part by this project will be reported below. The loss in efficiency of COD removal with nitrogen-lean activated sludges was evaluated under conditions of variable substrate, variable solids wasting, and variable forms of nitrogen supplement.

a. Influence of Substrate

Five sets of complete mixing activated sludge pilot plants were operated on K Acetate, K Lactate, Na Oleate, Glucose and Starch. All systems had a volumetric loading of 60 lbs COD/1000 cu ft/d and were operated such that wide ranges of percentage nitrogen in the mixed liquor volatile solids existed at various times. The COD reduction under these varying conditions was then determined. Plots of per cent nitrogen in the mixed liquor volatile solids vs. per cent COD remaining in solution are given in Figure 15 to 19. Per cent COD remaining in solution is used instead of COD concentration remaining in solution because at the present

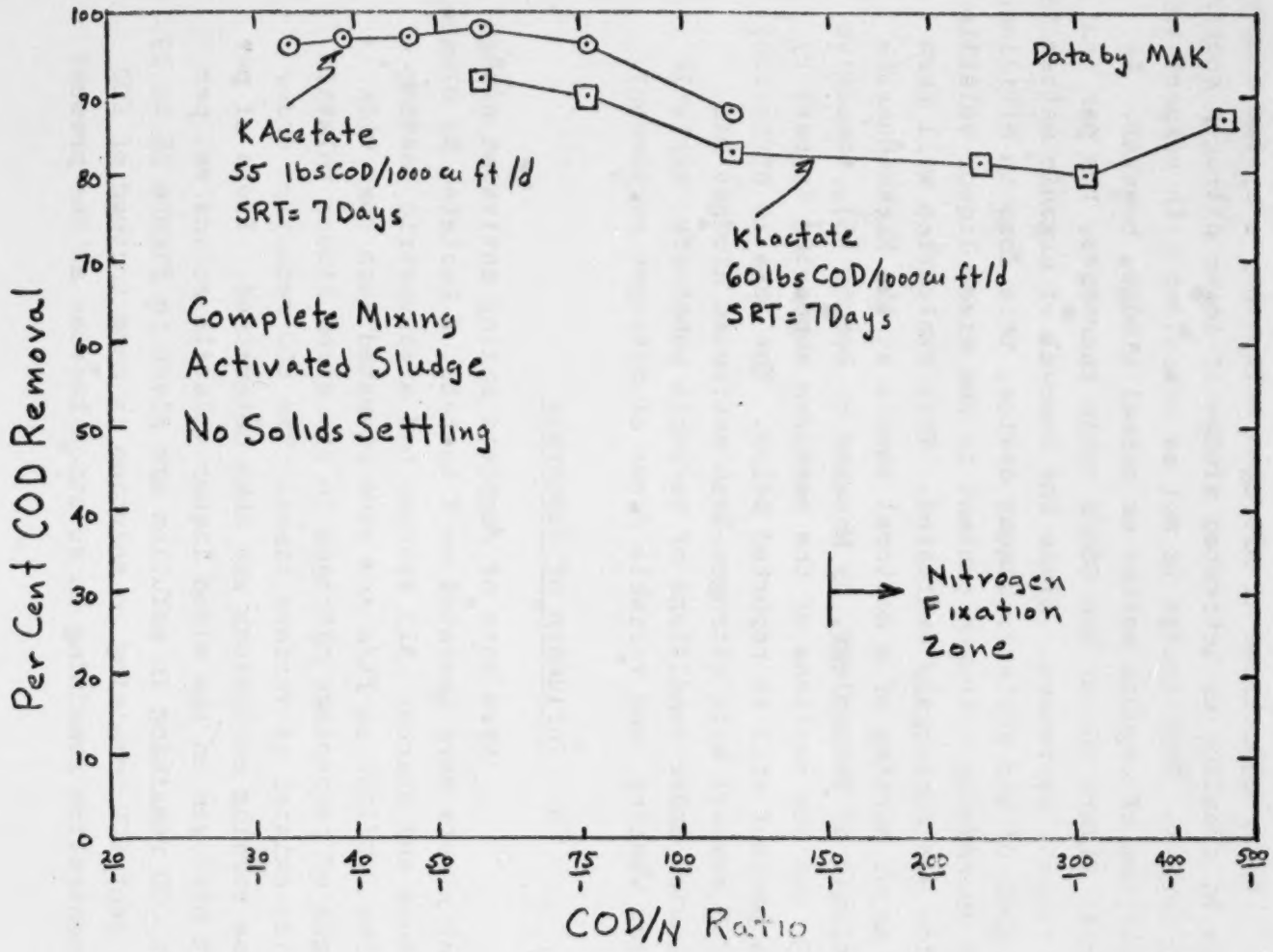


Figure 14 Influence of Nitrogen Fed on COD Removal

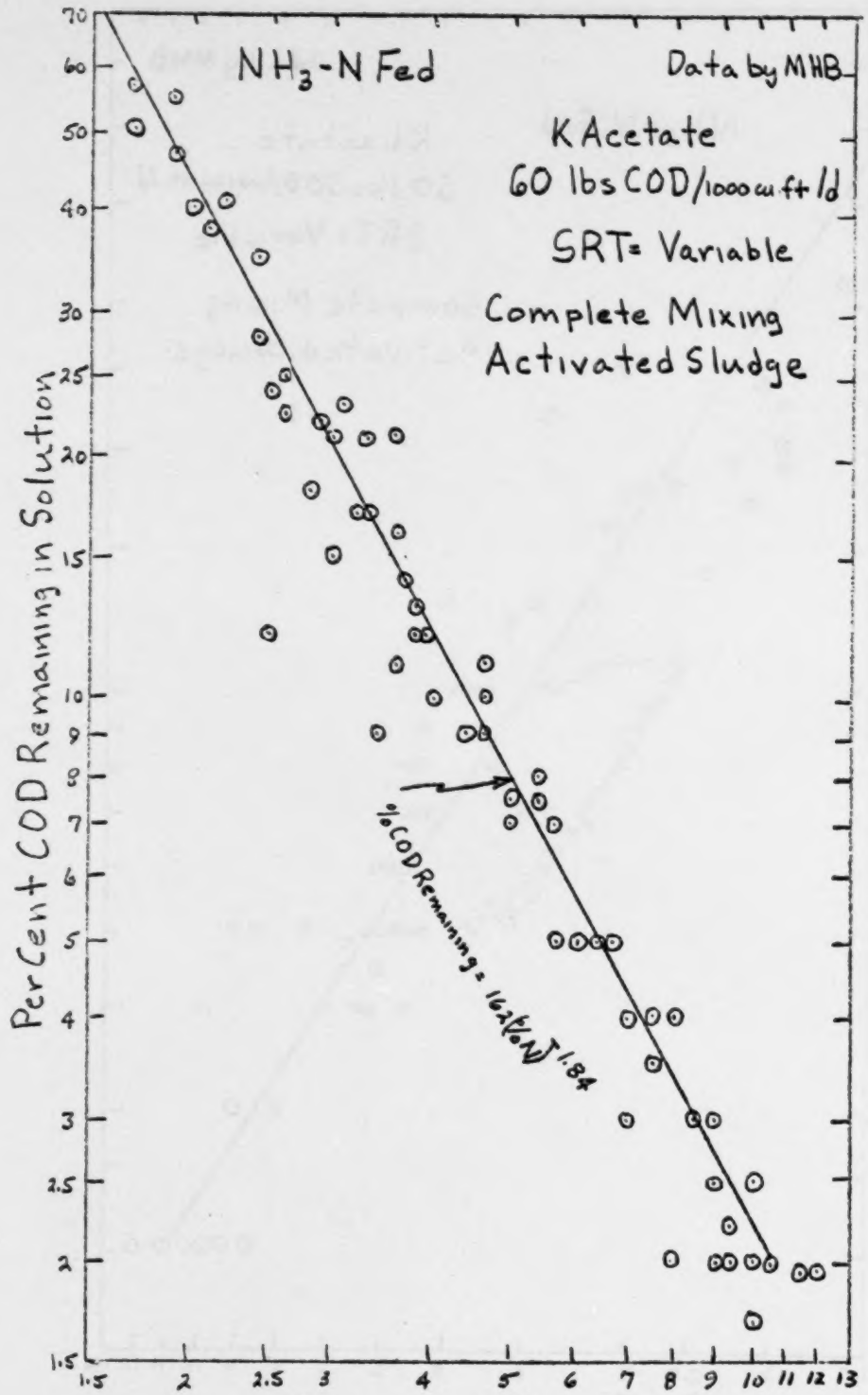


Figure 15 Nitrogen Effect - K Acetate

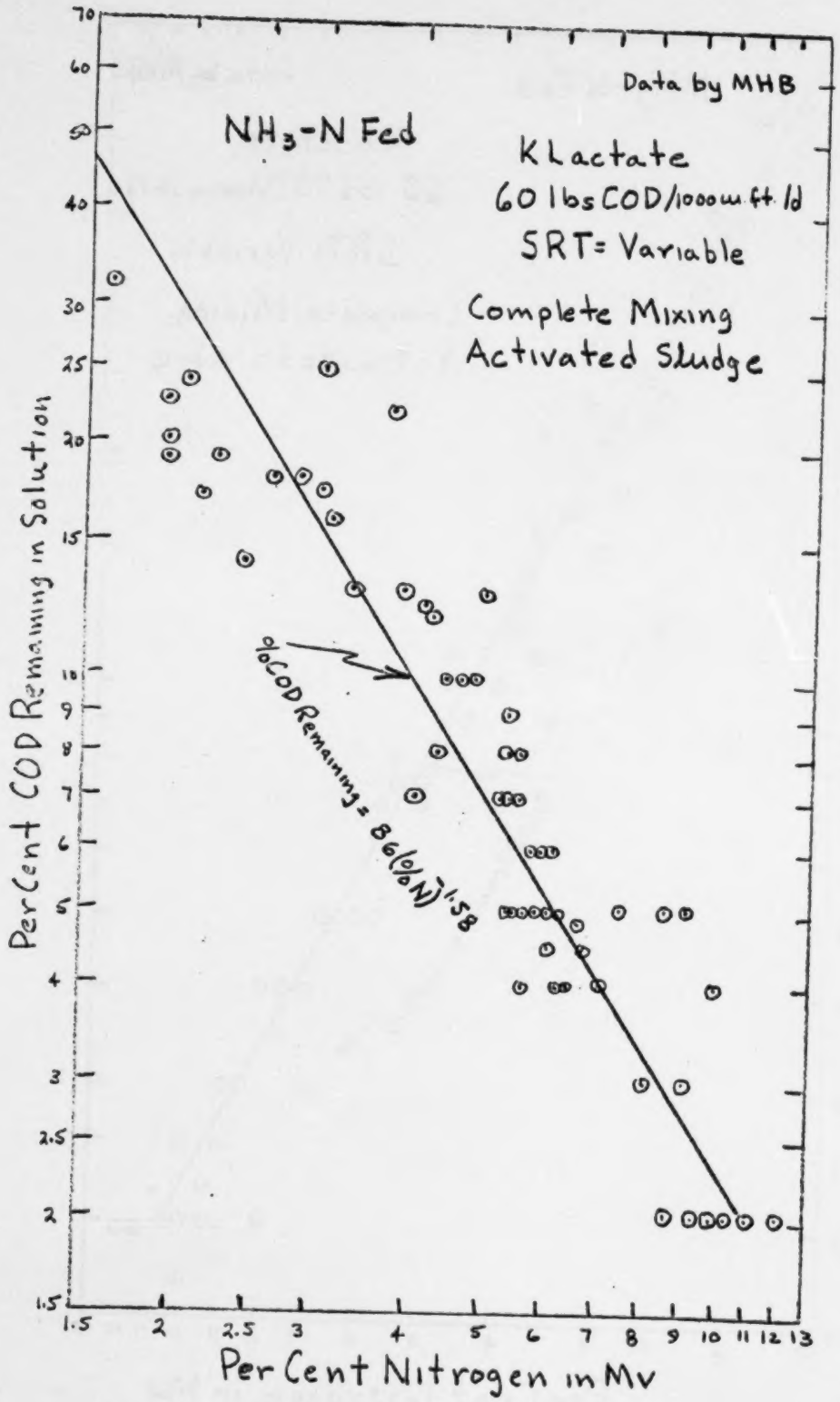


Figure 16 Nitrogen Effect - K Lactate

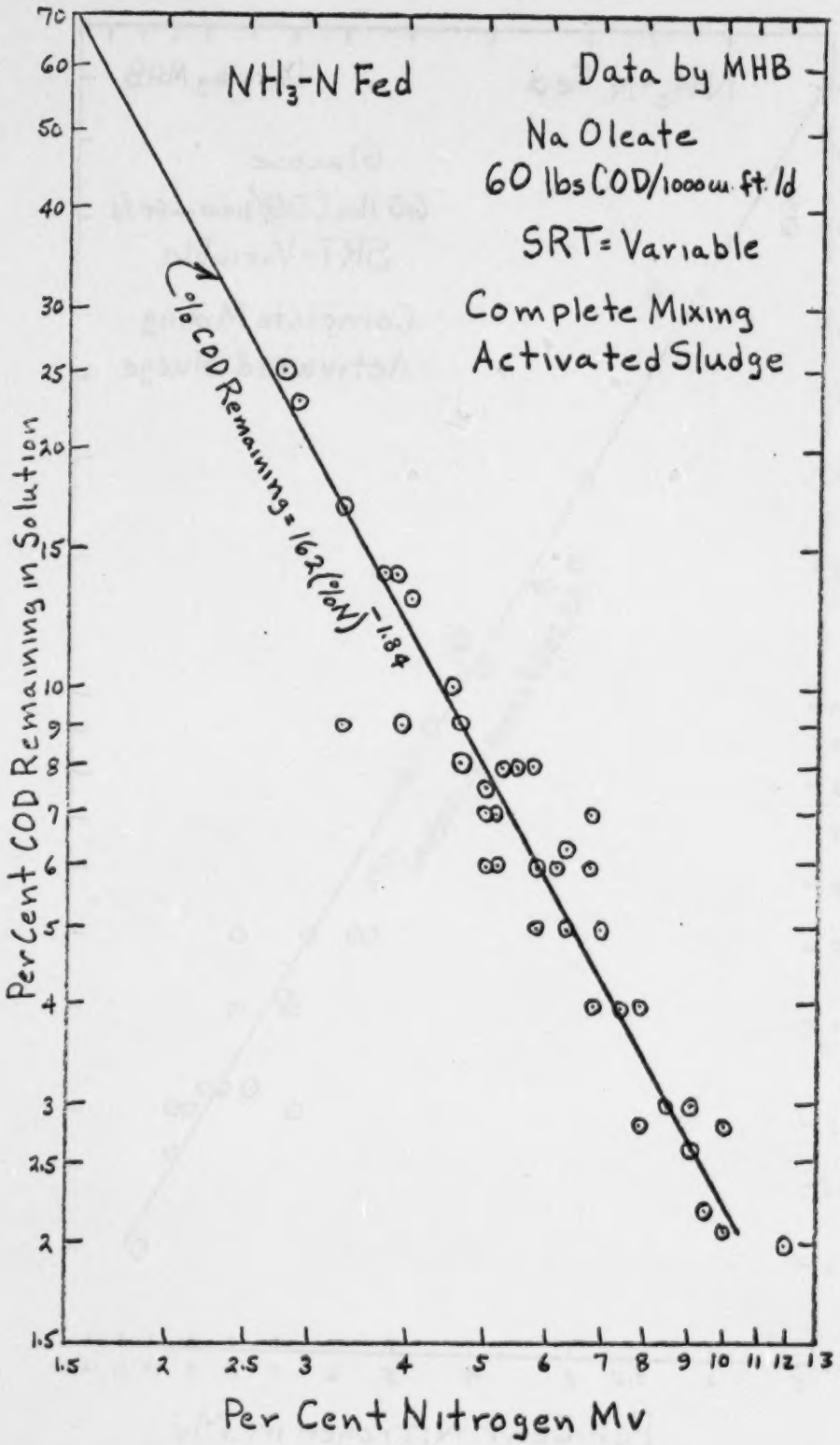


Figure 17 Nitrogen Effect - Na Oleate

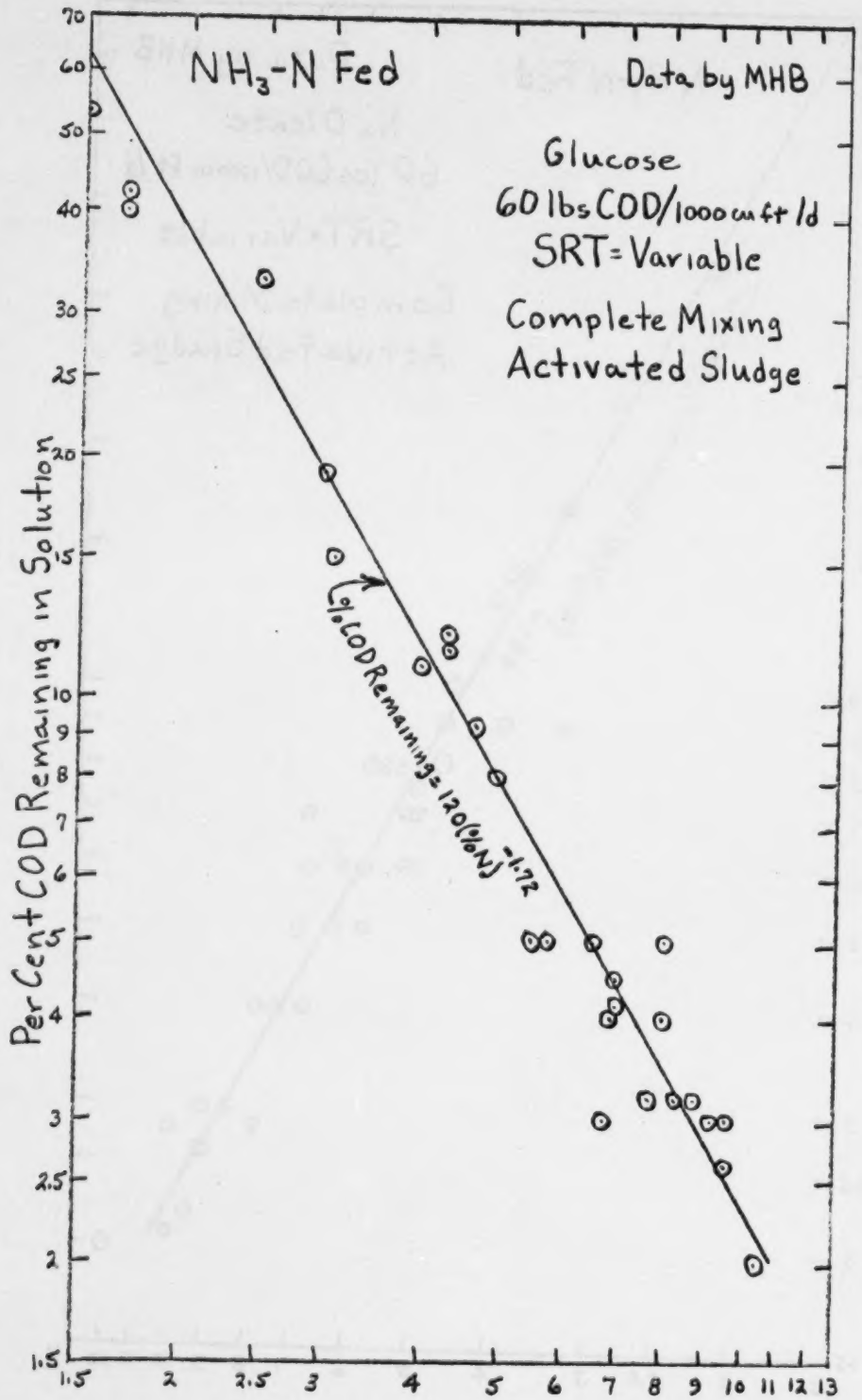


Figure 18 Nitrogen Effect - Glucose

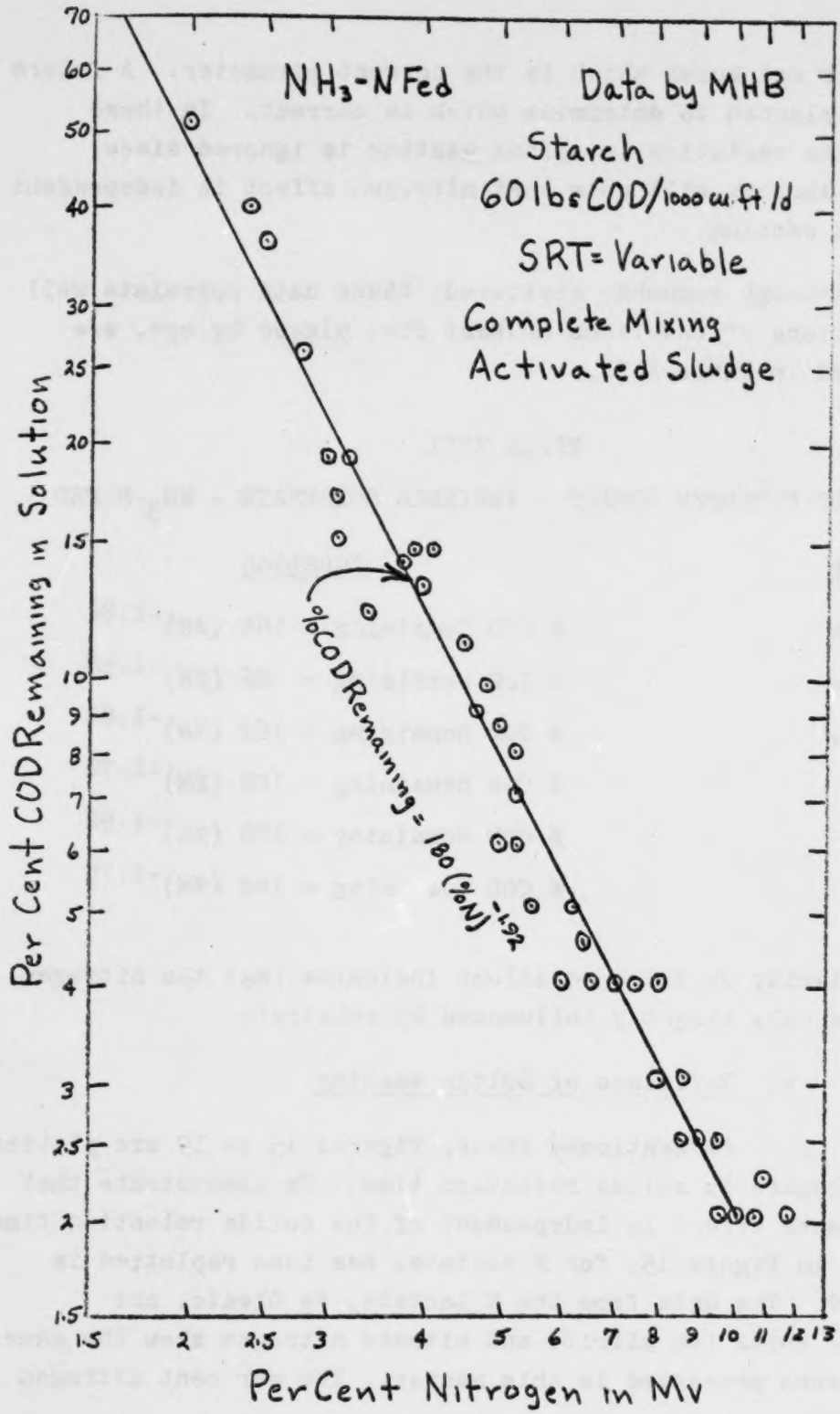


Figure 19 Nitrogen Effect - Starch

time it is not known which is the correct parameter. A future study is planned to determine which is correct. In these figures the variation in solids wasting is ignored since the next section will show that nitrogen effect is independent of solids wasting.

Although somewhat scattered, these data correlate well. The equations of the lines of best fit, placed by eye, are summarized in Table XIII.

TABLE XIII

SUMMARY OF NITROGEN EFFECT - VARIABLE SUBSTRATE - NH_3-N FED

Substrate	Equation
K Acetate	% COD Remaining = 162 $(\%N)^{-1.84}$
K Lactate	% COD Remaining = 86 $(\%N)^{-1.58}$
Na Oleate	% COD Remaining = 162 $(\%N)^{-1.84}$
Glucose	% COD Remaining = 120 $(\%N)^{-1.72}$
Starch	% COD Remaining = 180 $(\%N)^{-1.92}$
Average	% COD Remaining = 142 $(\%N)^{-1.78}$

The similarity of these equations indicates that the nitrogen effect is only slightly influenced by substrate.

b. Influence of Solids Wasting

As mentioned above, Figures 15 to 19 are plotted without regard to solids retention time. To demonstrate that the nitrogen effect is independent of the solids retention time, the data in Figure 15, for K Acetate, has been replotted in Figure 20. The data from the K Lactate, Na Oleate, and K Acetate units fed nitrite and nitrate nitrogen show the same pattern when presented in this manner. The per cent nitrogen

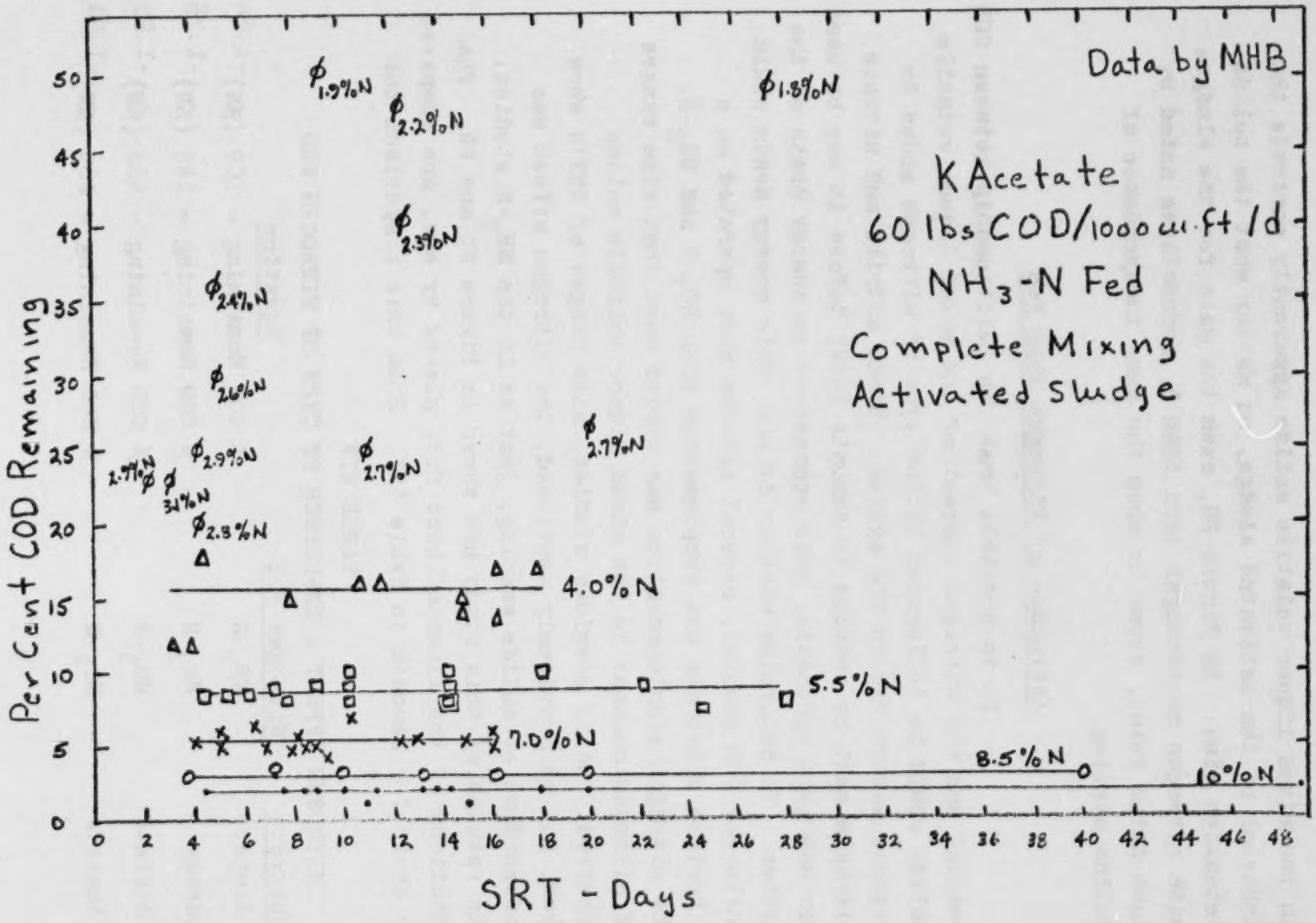


Figure 20 Nitrogen Effect - Influence of Varying SRT

in the mixed liquor volatile solids apparently controls the behavior of the activated sludge, no matter what the solids retention time. In Figure 20, even the data for the sludges with nitrogen percentages less than 4, percentages noted by each datum point, seems to show the same independence of solids wasting.

c. Influence of Nitrogen Form Fed

It is possible that the relationship between COD removal and the nitrogen content of the mixed liquor volatile solids could be influenced by the type of nitrogen added to organic matter fed to the system. Since nitrite and nitrate nitrogen must be reduced to ammonia level before it may be used for new cell synthesis, this represents an energy drain on the system. To determine whether or not this energy drain would influence COD removal, several systems were operated on a K Acetate substrate and supplemented with NO_3-N and NO_2-N. The nitrogen supplementation was varied such that wide ranges of nitrogen content in the mixed liquor volatile solids occurred. As in previous studies, wide ranges of SRT's were used, but as previously mentioned, the nitrogen effect was independent of solids wasting, just as in the NH_3-N studies. The results of this study are shown in Figure 21 and 22. The equations of the lines of best fit, placed by eye, are compared to those for ammonia in Table XIV. From this comparison the

TABLE XIV

NITROGEN EFFECT - INFLUENCE OF TYPE OF NITROGEN FED

Substrate	Nitrogen Fed	Equation
K Acetate	NH_3-N	% COD Remaining = 162 $(\%N)^{-1.84}$
Average	NH_3-N	% COD Remaining = 142 $(\%N)^{-1.78}$
K Acetate	NO_3-N	% COD Remaining = 460 $(\%N)^{-1.95}$
K Acetate	NO_2-N	% COD Remaining = 280 $(\%N)^{-1.90}$

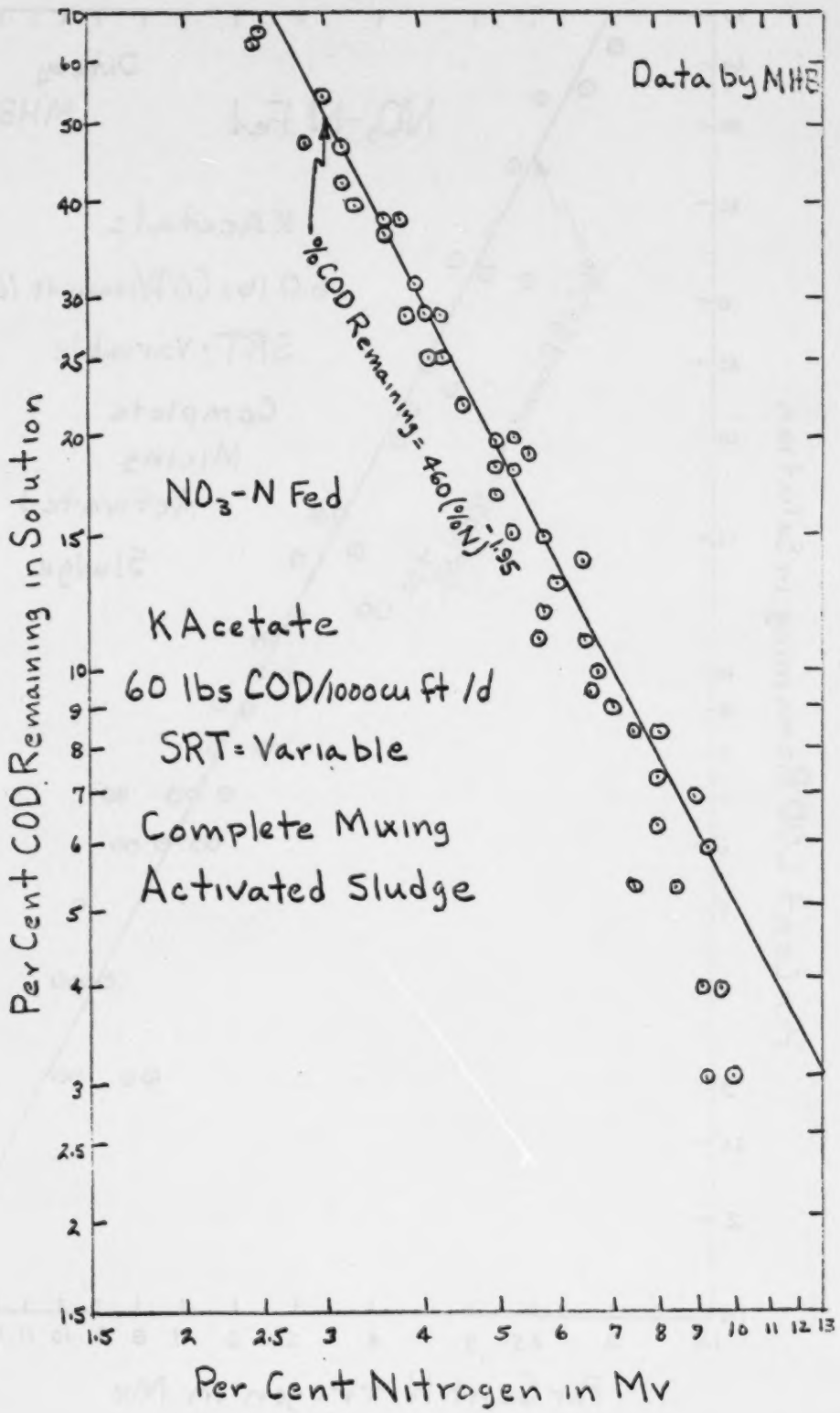


Figure 21 Nitrogen Effect - NO_3-N

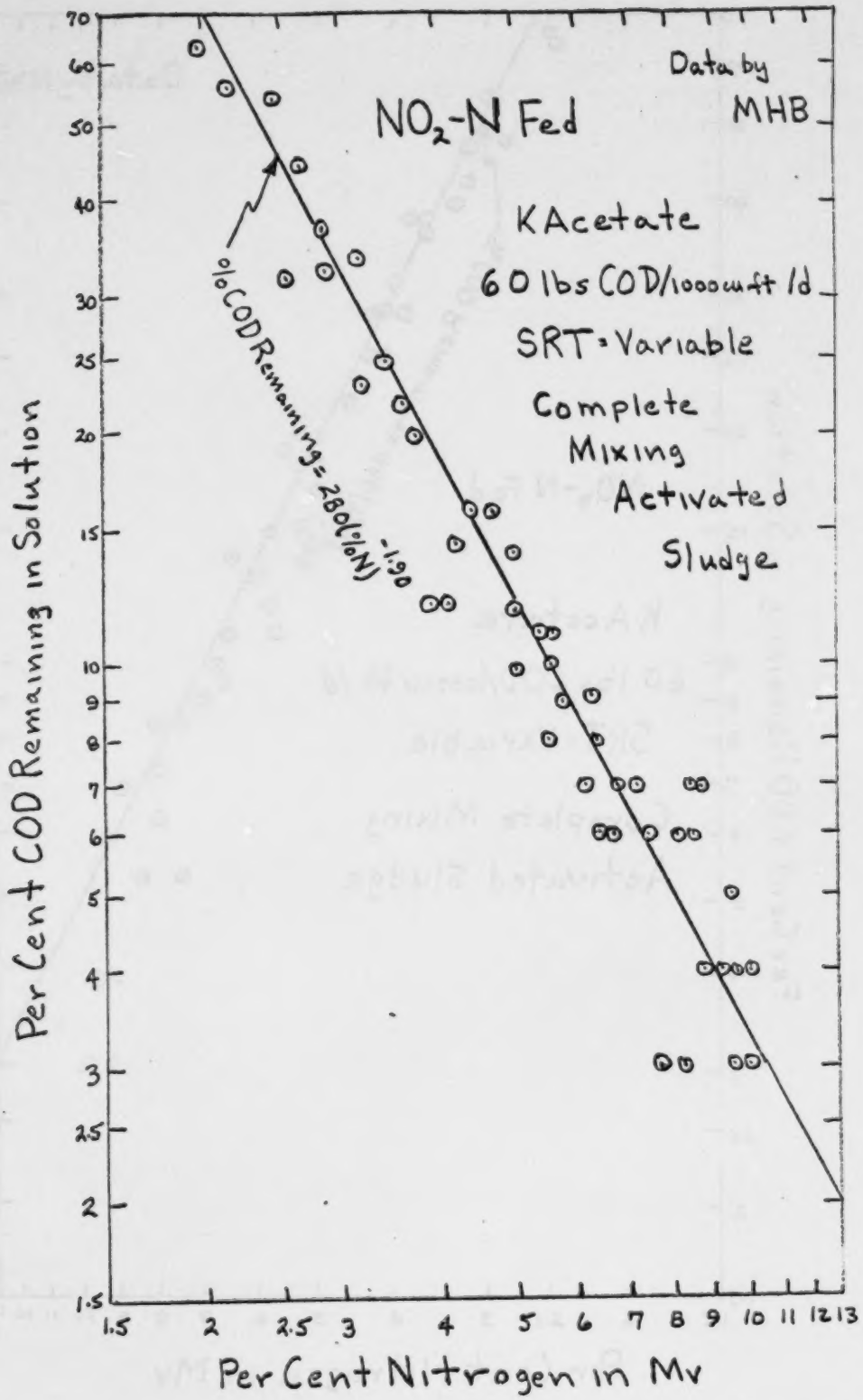


Figure 22 Nitrogen Effect - NO_2-N

influence of the extra energy drain caused by the reduction of nitrates and nitrites is seen. For a given nitrogen content, the "per cent COD remaining" would be higher for nitrites fed than for ammonia fed, and higher for nitrates fed than for nitrites fed. This decrease is in direct correlation with the energy requirements for synthesis of new cells. Use of ammonia nitrogen requires the least energy, nitrate nitrogen the most energy and nitrite nitrogen an intermediate quantity of energy, for synthesis.

d. Influence of Volumetric Loading

A future study to determine the influence of volumetric loading on the nitrogen effect and the determination as to whether the "per cent COD remaining in solution" or "COD concentration remaining in solution" is the correct dependent variable to correlate with the nitrogen content in the mixed liquor volatile solids is planned. This, as well as the changes which occur in a sludge at lower nitrogen content which causes it to be less efficient with respect to organic removal, will be included in Bechir's final report (5).

3. Nitrogen Fixation

a. Conventional Activated Sludge

A complete study of the possibility of supplementing a nitrogen deficient organic waste with atmospheric nitrogen fixed into the system by nitrogen fixing microorganisms was performed by Kaylor (6) for the degree of Master of Science in Sanitary Engineering, Massachusetts Institute of Technology, 1961. His work was partially supported by this project. In brief, his results showed that several nitrogen-free organic substrates could be metabolized in a batch-fed pilot plant operated with a very long solids retention time. Kaylor's systems were seeded

initially with the nitrogen-fixing bacteria, Azotobacter sp., but were operated for about one year with very little control before Kaylor's study began. During this time the activated sludge had lost its original characteristics and had become quite filamentous and quite black. It was active and settled very well, however. The available activated sludge was divided and fed various pure organic substrates (Na Acetate, Na Lactate, Na Oleate, Glucose, Na Benzoate, Starch) without nitrogen. In all cases, except the starch substrate, the concentration of mixed liquor volatile solids rose, as did the organic nitrogen in the activated sludge, the latter indicating true nitrogen fixation. The removal of organic matter was rapid and complete during this experiment. An attempt to start a second set of systems using soil seed met with failure, however. The rate of nitrogen fixation averaged 6.4 mg of N fixed per gm COD removed for all substrates, with a range of 5.8 to 7.7.

b. Complete Mixing Activated Sludge

During the operation of six continuously-fed pilot plants on a K Lactate substrate, SRT 7 days, with COD/N ratios from 57/1 to 460/1, nitrogen fixation began spontaneously in the systems with COD/N ratios of 230/1, 310/1 and 460/1. This was evidenced in two ways. One, as seen in Figure 13, the nitrogen discharged from these systems was continuously greater than the nitrogen added. Two, the soluble COD reduction declined in these units, then recovered as nitrogen fixation started. This is seen in Table XV. Figure 13 shows a fixation rate of 3.7, 6.8, and 8 mg N fixed per gm COD removal for the three systems respectively. These systems were operated without settling.

In order to determine if this spontaneous beginning of nitrogen fixation could be repeated, new activated sludges were

TABLE XV

SUMMARY OF COD REMOVAL FROM K LACTATE UNITS

Week	COD/N, 230/1 % COD Red.	COD/N, 310/1 % COD Red.	COD/N, 460/1 % COD Red.
2	90	83	79
3	83	57	47
4	70	41	33
5	61	49	33
6	76	64	51
7	85	85	83

started on the following three substrates, K Acetate, K Lactate, and Na Oleate. Five units were started on each substrate, each with a COD/N ratio of 25/1. When the systems were working well, the COD/N ratios were changed on four of the units in each series so that the final COD/N ratios were: 25/1, 100/1, 200/1, 400/1, and infinity (that is, no nitrogen added). This change was gradual and took place over a three-week period. These systems were operated a fairly long SRT's, 20 to 30 days, and were tested for 11 weeks. The units operated fairly well, the mixed liquor volatile solids concentration rose in all systems, the organic matter removal which fell after the change in ratio, see Table XVI for K Acetate system, recovered and remained good during the remainder of the test, and a nitrogen balance on the COD/N ratio = infinity systems showed an erratic, but generally positive rate of nitrogen fixation. Table XVII summarizes the average rate of nitrogen fixation in these systems.

While the operation of these systems looked good, the main problem encountered was with the bulky mass of the activated sludge which developed. Although systems such as this could operate on a laboratory scale, a field trial is necessary before the principle of nitrogen fixation can be accepted as a practical process useful for treatment of nitrogen deficient wastes in the field.

TABLE XVI

SUMMARY OF COD REMOVAL FROM K ACETATE UNITS

Data by MHB

Day After Reduction of Nit.	COD/N, 100/1 % COD Red.	COD/N, 200/1 % COD Red.	COD/N, 400/1 % COD Red.	COD/N, Inf. % COD Red.
15	96	96	94	93
18	98	76	91	96
21	-	95	93	97
24	96	93	92	95
27	87	63*	90	95
30	58*	63*	79	94
33	62	90	48	86
36	95	94	39*	87
39	94	95	49	85
42	96	93	68	-
45	95	94	89	72
48	94	96	93	71
51	96	95	93	50
54	-	-	-	39*
57	-	-	-	63
60	-	-	-	90
63	-	-	-	92
66	-	-	-	94

All units good through day 78

*Day of poorest operation

TABLE XVII

SUMMARY OF NITROGEN FIXATION RATES

Data by MHB

Substrate	Average Rate of Nitrogen Fixation mg N Fixed per gm COD Removed
K Acetate	22
K Lactate	12
Na Oleate	7.5

Note: These rates are for the activated sludges fed no nitrogen.

A second experiment was attempted in which activated sludges which had been fed ample quantities of nitrogen were suddenly completely starved for nitrogen in the feed solution, rather than slowly withdrawing the nitrogen over a three-week period. In this test the per cent nitrogen in the mixed liquor volatile solids quickly dropped to one and the systems died, with no indication of the onset of any nitrogen fixation. This method of stimulating spontaneous nitrogen fixation was a failure.

4. Summary and Discussion

When an activated sludge is restricted in the quantity of nitrogen added with the organic matter fed, the amount of the organics removed which becomes resultant solids goes up, but the nitrogen content of these solids goes down. By this method the system conserves the nitrogen added as much as possible, and keeps the nitrogen loss per unit time to a minimum. This nitrogen-lean activated sludge is not as efficient as an activated sludge containing ample nitrogen, however. This loss in efficiency of COD removal is related to the per cent nitrogen in the mixed liquor volatile solids and this relationship is fairly independent of the substrate being metabolized and is independent of the solids retention time being employed. This relationship is strongly influenced by the form in which the nitrogen in added - ammonia, nitrite, or nitrate - and for future studies will determine the influence of volumetric loading. Although at the present time the exact reason for the decline in efficiency in the nitrogen-lean sludges is not known, the percentage of organic nitrogen in the mixed liquor volatile solids is apparently a good parameter to indicate the general health of a system.

The use of nitrogen fixation as a mechanism of nutrient supplementation was generally successful on a laboratory scale. The sludges developed were generally filamentous in nature

and sometimes caused a problem due to lack of setting. A field trial of this approach would better evaluate its usefulness. A nitrogen fixation rate of 10 mg of N fixed per gm of COD removed is a COD/N ratio of 100/1 which would seem adequate for certain operating conditions. Nitrogen fixation rates approaching this seem possible.

F. Related Studies

1. Determination of Active Mass

One problem which plagues sanitary engineers, both in the laboratory and in the field, is the determination of the fraction of the mixed liquor volatile solids which are live microorganisms actively metabolizing organic matter - that is, the active mass. One suggestion made by McKinney and Symons (7) was to measure the initial rate of oxygen uptake of an unfed activated sludge sample. This rate could then be compared to the rate of oxygen uptake of a sludge composed entirely of active organisms, and the quantity of active mass in the original sample calculated. The problem in using this method is the determination of the rate of oxygen uptake of active organisms.

If one had an active mass, only a portion of this could be degraded to carbon dioxide and water, part would turn into inert mass. Estimates of this degradable portion of active mass vary; McKinney and Symons (7) suggest 80 per cent, McCarty and Broderson (3) report 75 or 81 per cent, Kountz and Forney (8) gave 77 per cent, while data presented earlier in this report (Table X) show 82 per cent of newly synthesized active mass in complete mixing activated sludge is potentially degradable. The degradable portion of the active mass will take up oxygen at a certain rate under unfed conditions and the suggestion has been made that this rate would be independent of the nature or history of the sludge. This oxygen uptake

rate has been variously reported as 20 mg/l/hr/1000 mg/l of active mass (7), 8 mg/l/hr/1000 mg/l of active mass (3), 27 mg/l/hr/1000 mg/l of active mass - based on a mass decline of 0.58 per day reported earlier in this report for complete mixing activated sludge (Table XI).

Two attempts were made to measure this rate directly; one with pure cultures of bacteria, and the other with complete mixing activated sludge grown under such high wasting (SRT = 0.25 days) that the organisms were very close to 100 per cent active. In the pure culture studies, masses of organisms were grown on nutrient broth media, harvested, concentrated, washed twice in buffered distilled water, suspended in this media, and the oxygen uptake measured with a Warburg Respirometer. The organisms used were: E. coli, S. lutea, B. subtilus, A. aerogenes, and mixtures of the four grown separately. The activated sludges studied were doubly washed before being placed in the Warburg Respirometer in buffered distilled water.

The oxygen uptake was measured between hours two and three and the active mass was taken as the volatile solids mass in the Respirometer flask. The data are summarized in Table XVIII.

The pure cultures and mixed pure cultures, in general, gave rates lower than the activated sludges, possibly due to the erroneous assumption that the mass was completely active. In any event, this did not seem like a good method of evaluating activated sludge. The rates for the five activated sludges averaged 14.6 mg/l/hr/1000 mg/l of volatile mass with a standard deviation of 3.3 mg/l/hr/1000 mg/l of volatile mass. This rate is higher than reported by McCarty and Broderson (3), but lower than reported by McKinney and Symons (7), and earlier in this report, for active mass. It is interesting to note that data taken in a similar manner for conventional activated sludges with SRT's of 1.5 and

TABLE XVIII

SUMMARY OF INITIAL OXYGEN UPTAKE RATES FOR VARIOUS SAMPLES

Data by MWT

Sample	mg/l O_2hr/1000 mg/l of Volatile Mass (Active Mass)
E. coli	1.8
S. lutea	9.2
B. subtilus	13.1
A. aerogenes	4.8
Mixed Pure Cultures	8.6
Mixed Pure Cultures	14.0
Complete Mixing Activated Sludge	13.1
Complete Mixing Activated Sludge	14.8
Complete Mixing Activated Sludge	10.6
Complete Mixing Activated Sludge	19.6
Complete Mixing Activated Sludge	15.0

1.25 days had oxygen uptake rates of 15.3 and 19.0 mg/l/hr/1000 mg/l of volatile mass.

McKinney and Symons (7) presented a formula for the oxygen uptake of an active mass as $O_2 = 1.12\ Mao\ (1-2^{-0.02t})$ for t in hours. Based on the above data the formula would be $O_2 = 1.15\ Mao(1-e^{-0.015t})$. Unfortunately the data taken for the complete mixing activated sludge samples does not follow an equation of this form as shown by Figure 23. The reason for this is not known at this time.

A third attempt was made to measure the active mass of an activated sludge by noting the rate of oxygen uptake of various systems which were felt to be nearly 100 per cent active when fed high concentrations of glucose. This study

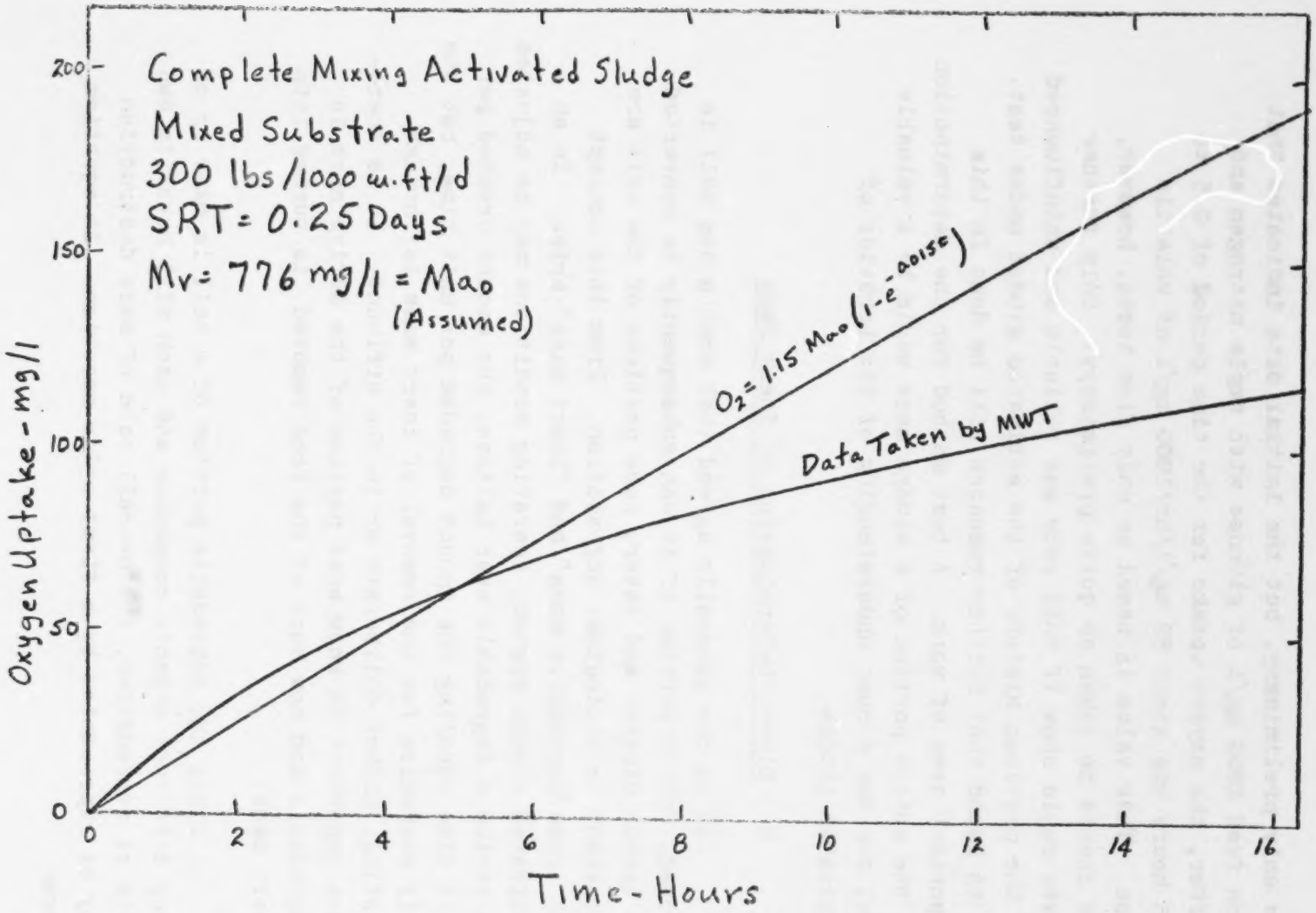


Figure 23 Comparsion of Theoretical and Actual Oxygen Uptake of an Active Mass

was only preliminary, but the initial data indicated that when feed 2000 mg/l of glucose with ample nitrogen and buffer, the oxygen uptake for the time period of 0.5 to 1.5 hours was about 50 mg/l/hr/1000 mg/l of volatile mass. This value is based on only five tests, however, and should be taken as quite preliminary. Only further tests would show if this rate was reliable and uninfluenced by the previous history of the activated sludge under test. It is hoped that further research will be done in this important area of work. A test method for the determination of the active portion of a sludge mass would be a valuable tool for the proper understanding of the behavior of activated sludge.

2. Direct Determination of Inert Mass

It is now generally agreed that when a new cell is formed, only a portion of it can subsequently be converted to carbon dioxide and water, some portions of the cell are resistant to biological degradation. From this concept the terms "degradable mass" and "inert mass" arise. In an activated sludge system, operating conditions may be adjusted to strike a degradable mass balance, the amount created per unit time equaling the amount degraded per unit time, but the only mechanism for the removal of inert mass is through wasting, either deliberate or in the effluent. It is therefore important to know what portion of the active mass is degradable and how much of the food removed is turned into inert mass.

Since the degradable portion of a cell is made up of many different organic compounds and each will have its own rate of degradation, the overall rate of mass destruction may be represented by a first order reaction. In equation form:

$$Mvt = Mdo(10^{-kt}) + Mit \qquad (1)$$

Where: Mvt is the volatile solids concentration at time "t"
Mdo is the degradable mass concentration at time "0"
k is the rate constant
t is the time in days
Mit is the inert mass concentration at time "t"

This equation may also be written:

$$Mvt = Mao(Md/Ma)(10^{-kt}) + Mit \qquad (2)$$

Where: Mao is the active mass concentration at time "0"
Md/Ma is the portion of the active mass which is degradable

A third method of expressing this same concept is:

$$Mvt = (Mvo-Mio)(Md/Ma)(10^{-kt}) + Mit \qquad (3)$$

Where: Mvo is the mixed liquor volatile solids concentration at time "0"
Mio is the inert solids concentration at time "0"

Table XIX summarizes some of the data pertinent to this discussion.

TABLE XIX

SUMMARY OF DATA ON INERT MASS AND MASS DEGRADATION

Sample	$\frac{\text{Degradable Mass}}{\text{Active Mass}}$ Ratio	$\frac{Mi}{Fr}$ Ratio Mass/O_2 Equiv.	Rate of Degradable Mass Degradation 1/Day	k_{10} for t in days	Reference
Complete Mixing Activated Sludge	0.82	0.09	0.58 (Fed)	0.39	This report Table XI
Conventional Activated Sludge	0.73-0.77	0.16	0.21-0.29 (Fed)	0.11-0.15	This Report Table XI
Activated Sludge	0.75 or 0.81	0.09	0.18 (Fed)	0.09	(3)
Activated Sludge	0.80	0.19	0.43 (unfed)	0.25	(7)
Conventional Activated Sludge	0.60	0.12	0.25-0.33 (unfed)	0.13-0.18	(9)
Activated Sludge	0.77	0.07	--	--	(8)
Completely Active Mass	--	--	0.30 (unfed)	0.16	This report Previous Section

The following experiment was to check on these unfed data with a nearly completely active mass. The four cultures, E. coli, A. aerogenes, S. lutea, and B. subtilus were grown independently in a high concentration of nutrient broth for three days, aerated by shaking. After this time the cultures were washed, mixed, and placed on a wrist shaker in bacterial buffer water. The volatile solids concentration was then determined periodically over the next 24 days, with aseptic conditions being maintained. The results of this study are shown in Figure 24.

Since complete activity was assumed at the start of this test, Mio in equation 3 is zero and the inert mass in the system was the volatile mass concentration determined at the end of the test, Mvf. Therefore, the equation for the die-away of these bacterial could be written:

$$Mvt = (Mvo)\left[(Mvo-Mvf)/Mvo\right](10^{-kt}) + Mvf \qquad (4)$$

$$Mvt = (930)\left[(930-120)/930\right](10^{-0.18t}) + 120$$

This equation is plotted in Figure 24 as a comparison to the actual data. The data in the box should be compared with Table XIX.

These data in Figure 24 are for batch-fed pure cultures. A future study should be conducted using continuous-fed highly wasted activated sludge. Also the rates determined here are for unfed organisms. There is much disagreement related to the influence of feeding on the rate of solids destruction. Some recent work (10)(11)(12) indicates that the influence is small, but Ludzack,Krieger and Ettinger (13) reported that the nature of the feed influences the rate of solids destruction.

Finally, a companion study, identical to the one described above, but with one exception was carried out.

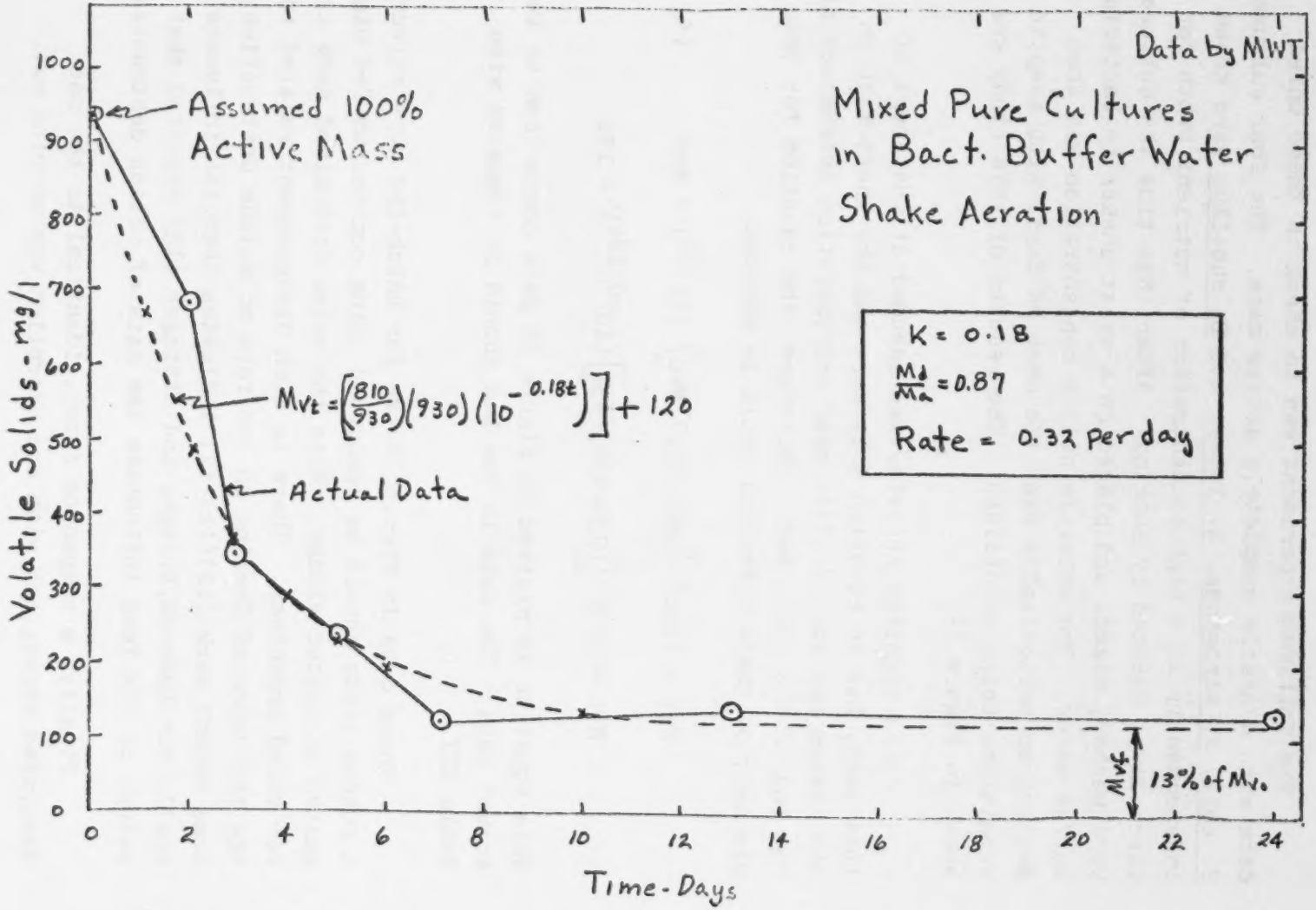


Figure 24 Die-Away Curve for Active Mass

This exception was the seeding of the flask at the start of the test with a mixed culture of rotifers purchased from the Carolina Biological Co. This system behaved almost identically with the one shown in Figure 24, indicating that the presence of predators had little influence on rate of solids destruction, at least under these circumstances.

3. Biological Destruction of Inert Mass

A definition of inert mass in an activated sludge system is the portion of biological mass which is resistant to biological degradation under existing conditions. The biological masses in activated sludge almost all contain some inert mass, the percentage depending on the amount of solids wasting (3). Figure 24 showed a degradation curve for a biological mass with very little, if any, inert mass at time zero. The degradation rate in this case was 0.32 per day. Studying twelve activated sludges grown with very little solids wasting for 12 weeks on either Na Acetate, glucose, or glycine (six as complete mixing activated sludge and six as conventional activated sludge), Washington, reported by Symons (2), found degradation rates from 0.09 to 0.02 per day, averaging 0.04 per day under unfed conditions. Kountz and Forney, also studying activated sludge grown under very low wasting solids conditions, reported a degradation rate of 0.02 per day under unspecified conditions. These low rates indicate the presence of a large percentage of inert mass in the mixed liquor volatile solids.

A question arose, however, as to how resistant to biodegradation this material really was. Was it completely inert, or was it just so slowly degraded as to be inert for all practical purposes? To investigate this, several activated sludges grown on a mixed substrate in continuously-fed pilot plants with very little solids wasting for several

weeks were mixed together and aerated for over one year. During this time, the mixed liquor volatile solids concentration was determined periodically. The results are shown in Figure 25.

Based on the initial mixed liquor volatile solids concentration, the initial degradation rate was 0.03 per day. Since this is about 10 per cent of the rate determined from Figure 24, the activated sludge was calculated to be 90 per cent inert mass at the start of the test. If 180 mg/l of active mass existed at the start of the test, and 82 per cent of it was degradable, 1820 - 0.82 (180) or 1670 mg/l of inert mass or potentially inert mass existed in the system at time zero. Since the degradable mass decayed at a rate of 0.32 per day, it was essentially gone by the tenth day, lower curve, Figure 25. Subtracting the degradable mass from the total mass, the rate of degradation of the inert mass was calculated as 0.023 per day. This low, but positive rate, indicates that the term, inert, is relative and that even the so-called inert mass is slowly biodegradable.

Some portions of the initial "inert" mass is biologically harder than other portions, however. This is shown by comparison of the middle curve with the actual data in Figure 25. A degradation rate of 0.023 per day means that only 0.01 of the original material remains after 200 days. This was not true as 21 per cent remained after 375 days. Over the last 100 days of the test the degradation rate calculated to be 0.0004 per day. This remaining material is quite, but not completely, resistant to biodegradation. It might even be largely made up of spores. Table XX summarizes additional data taken after 375 days of continuous aeration. The sludge, while not too active, was not dead.

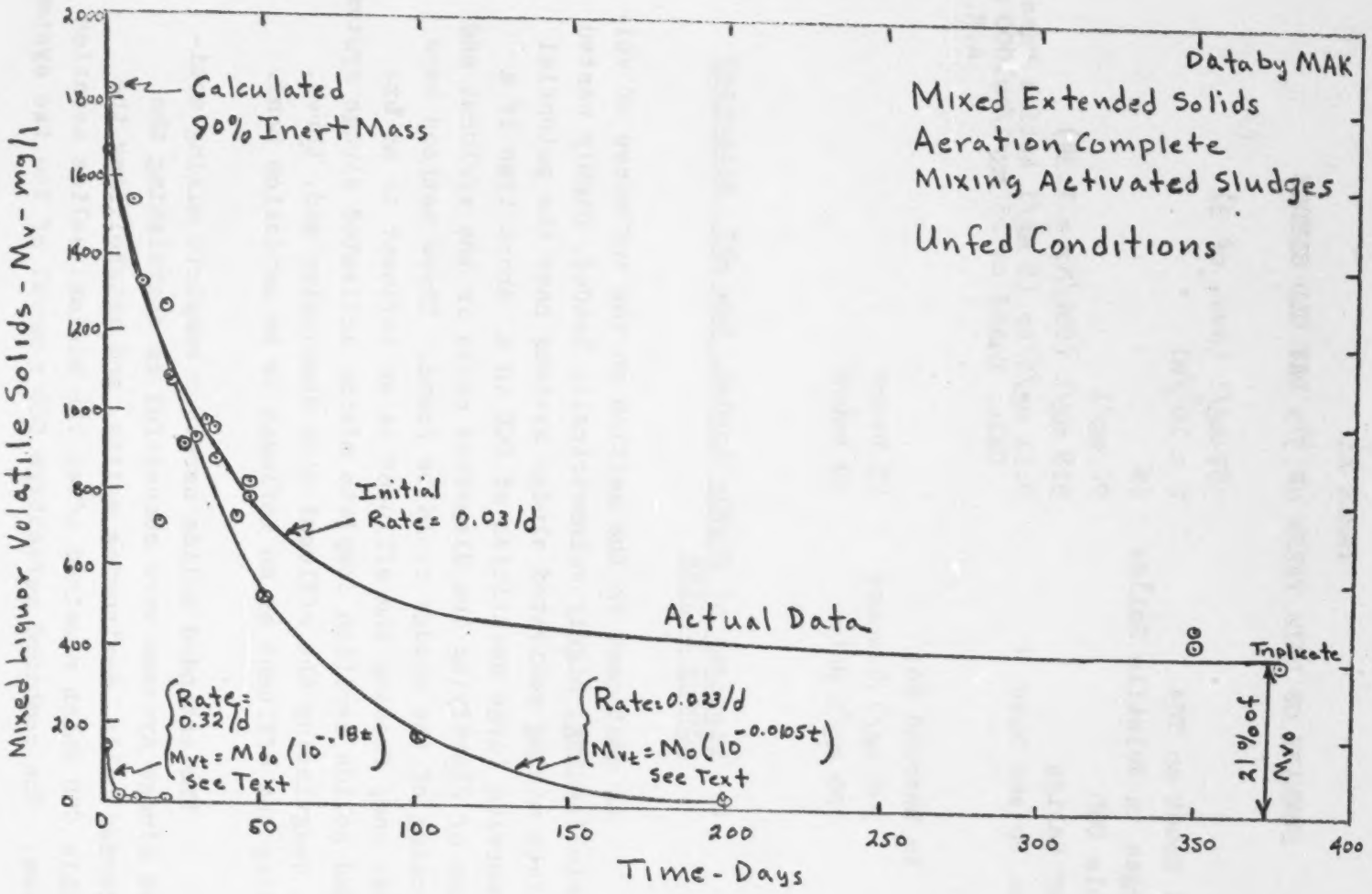


Figure 25 Die-Away Curve for Inert Mass

TABLE XX

SUMMARY OF DATA TAKEN ON 375 DAY OLD SLUDGE

Mv	387 mg/l (Ave. of 3)
Total Count on TGA	2×10^5/ml
Nitrogen in Volatile Solids	6%
Soluble COD	92 mg/l
COD of Solids	518 mg/l (COD/Mv = 1.34)
Oxygen Uptake Rate	0.11 mg/l/hr (8 mg/l Active Mass-Calc. Based on 15 mg/l/hr/1000 mg/l A.M.)
Time to Respond to:	
500 mg/l Glucose	75 hours
500 mg/l NaAc	50 hours

4. Disposal of Highly Loaded, Low SRT, Activated Sludge Solids

As mentioned in the section on the influence of volumetric loading, highly volumetrically loaded, highly wasted complete mixing activated sludge systems have the potential of removing large quantities of COD in a short time if a method of clarifying the dispersed cells in the effluent and disposing of the sludge could be found. Three methods were tried: one, feeding the effluent as an influent to an extended solids aeration complete mixing activated sludge system; two, coagulating the effluent with chemicals; and, three, feeding the effluent as an influent to an oxidation pond.

The extended solids aeration complete mixing activated sludge systems were successful in coagulating the dispersed cells, destroying solids, and metabolizing the soluble COD which remained after the minimal solids aeration system. The combined percentage COD removal of the two systems

in series was well over 90 for all volumetric loadings. Table XXI summarizes the operation of the systems with respect to solids desturction. The SRT of the secondary systems ranged between 10 and 15 days. Except for the first system which was so lowly loaded as to be atypical, the Mr/Fr ratios were at the minimum values possible, based on previous experiments. These systems worked quite well but future studies should be made to see to what extent the loadings on the secondary units could be increased, to cause further savings in tank volume.

TABLE XXI

SOLIDS DESTRUCTION IN COMBINED COMPLETE MIXING ACTIVATED SLUDGE SYSTEMS

Primary Vol Loading lbs COD/1000 cu ft/d	Sec. Vol. Loading lbs COD/1000 cu ft/d	Combined Mr/Fr Mass/O_2 Equivalent
61	11	0.21
152	25	0.07
313	39	0.09
584	83	0.09
620	124	0.08
860	127	0.12
925	184	0.07

Figures 26 to 31 show the results of chemical coagulation of the minimal solids aeration effluent, both with aluminum sulfate and with an organic coagulant. The coagulants were effective, but the dosages were high, probably because of the excessive phosphate buffer which was used along with organic substrates. The settled sludges were quite compact (Figures 28 and 31) but would need to be further dewatered before final disposal, possibly by incineration.

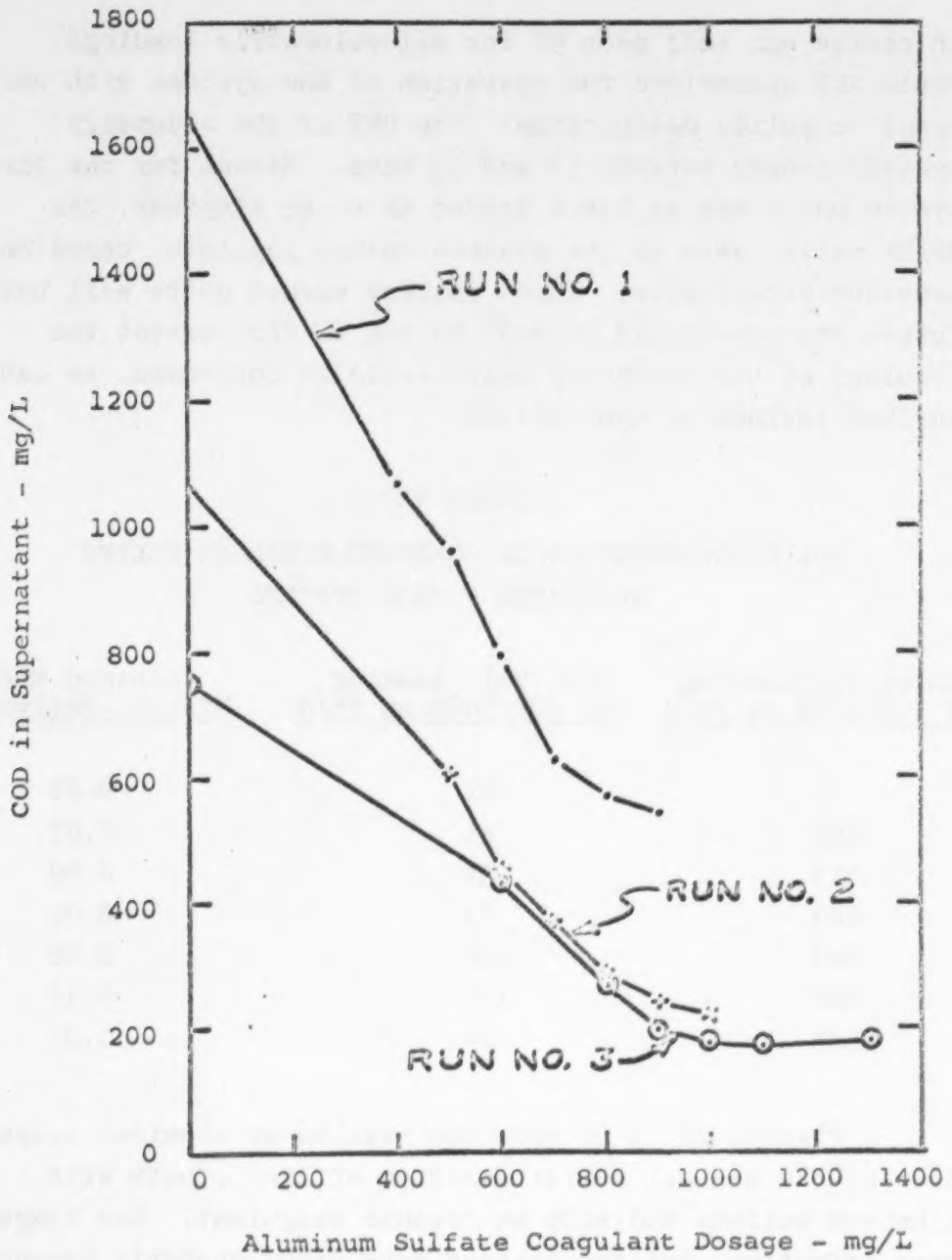


Figure 26 - DECREASE IN COD OF SUPERNATANT WITH ALUMINUM SULFATE COAGULANT

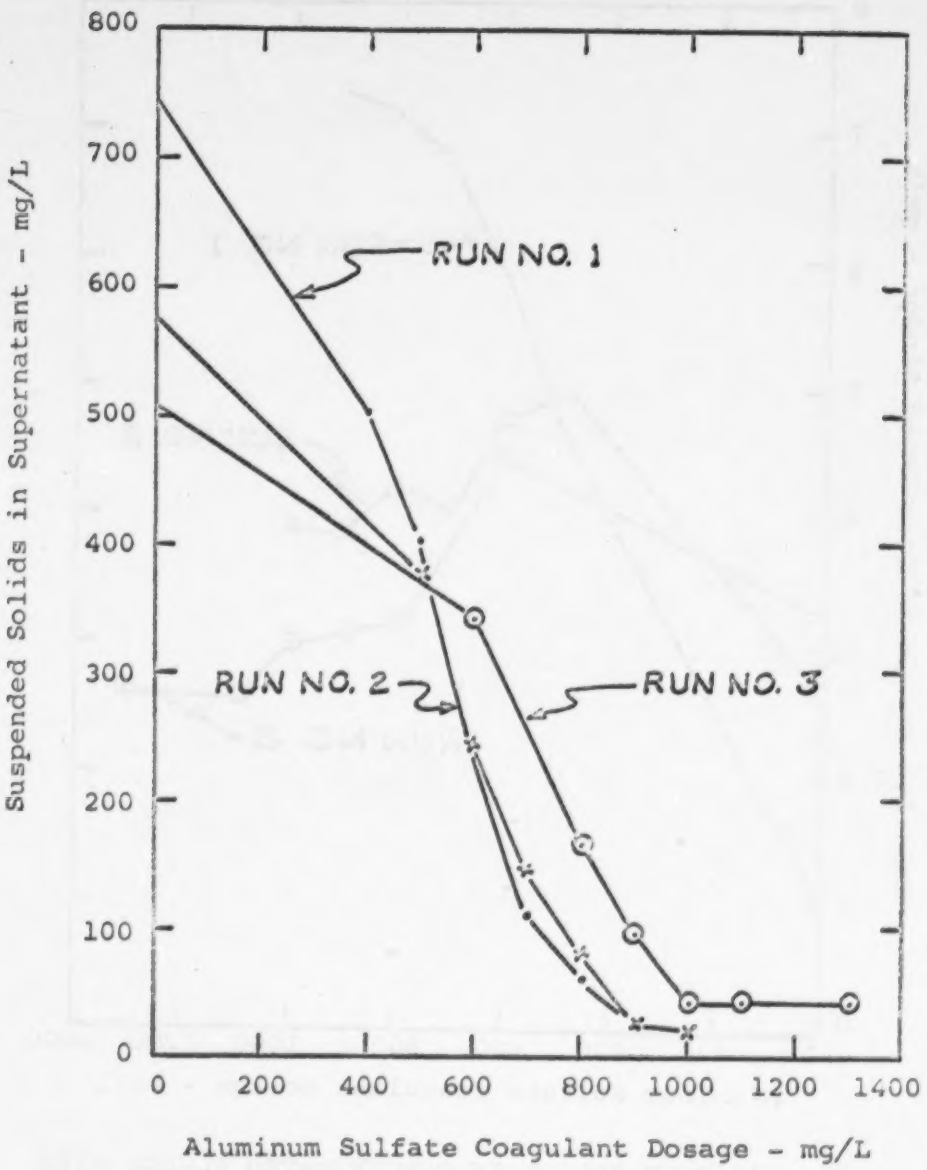


Figure 27 - CHANGE IN SUSPENDED SOLIDS IN SUPERNATANT WITH ALUMINUM SULFATE COAGULANT

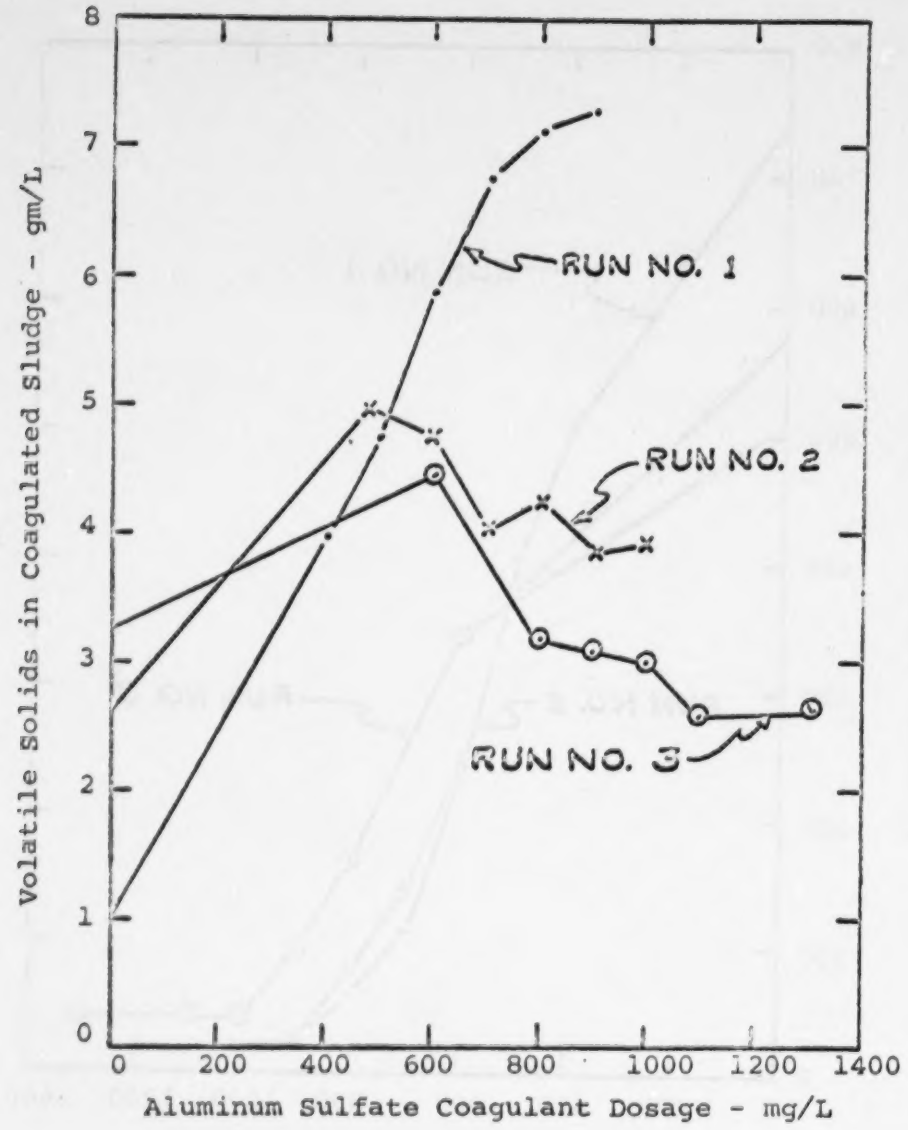


Figure 28- VOLATILE SOLIDS IN CONCENTRATED SLUDGE WITH VARYING DOSES OF ALUMINUM SULFATE COAGULANT

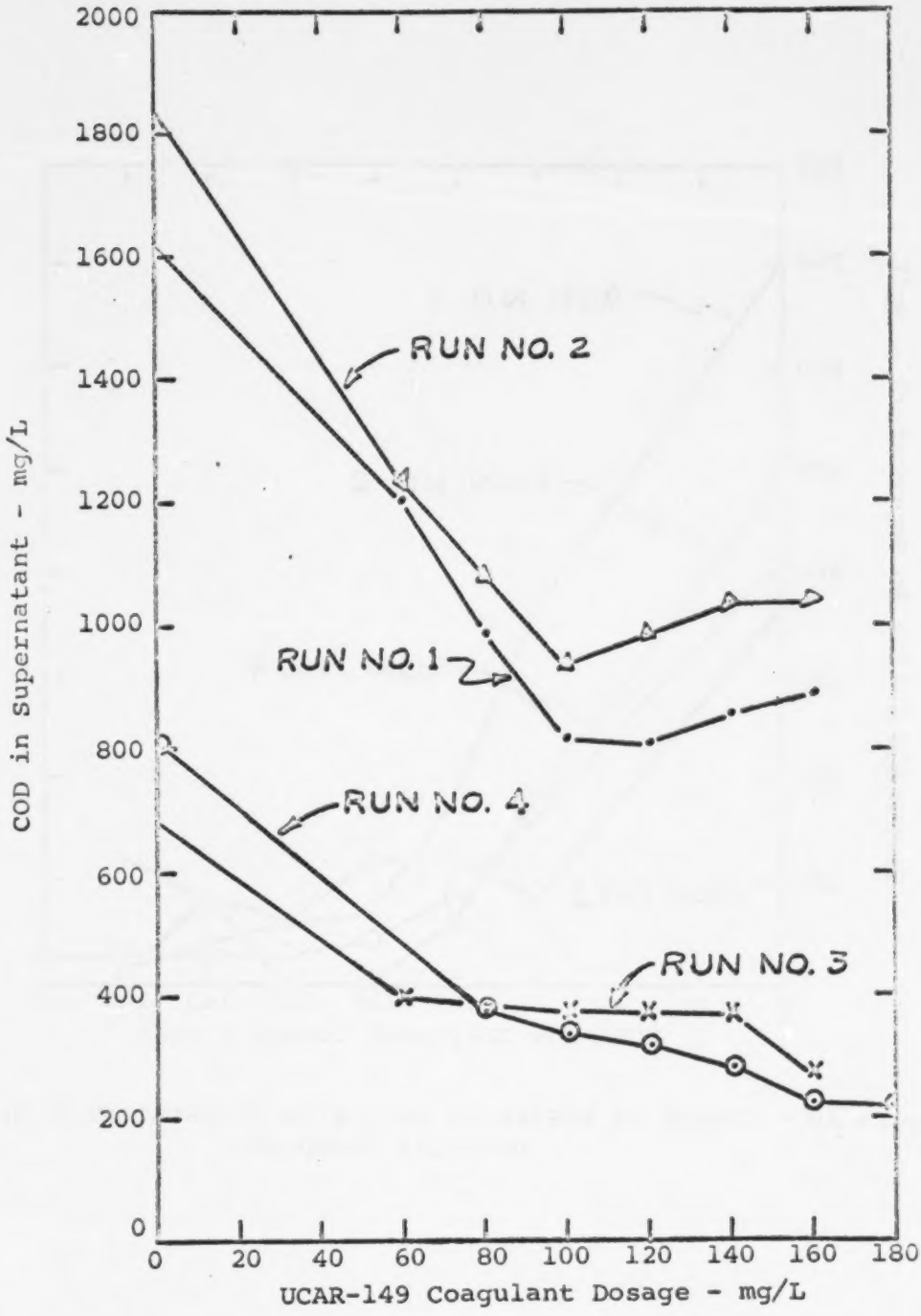


Figure 29 - CHANGE IN COD OF SUPERNATANT WITH UCAR-149 COAGULANT

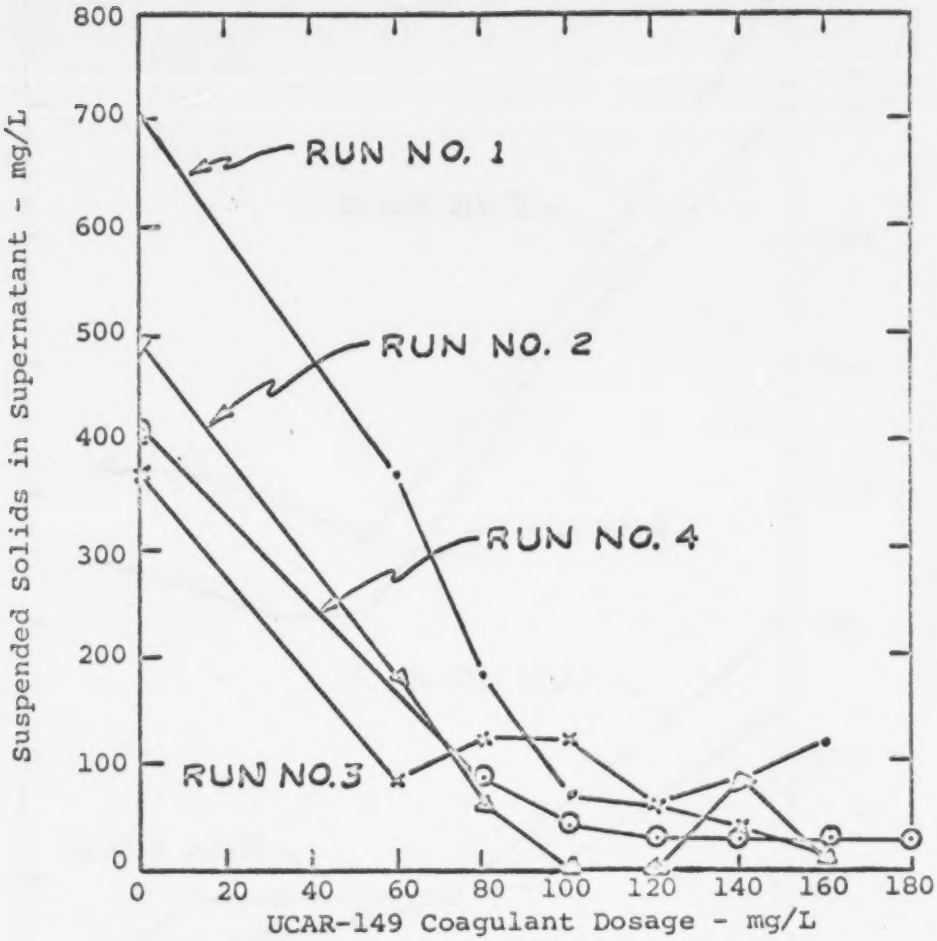
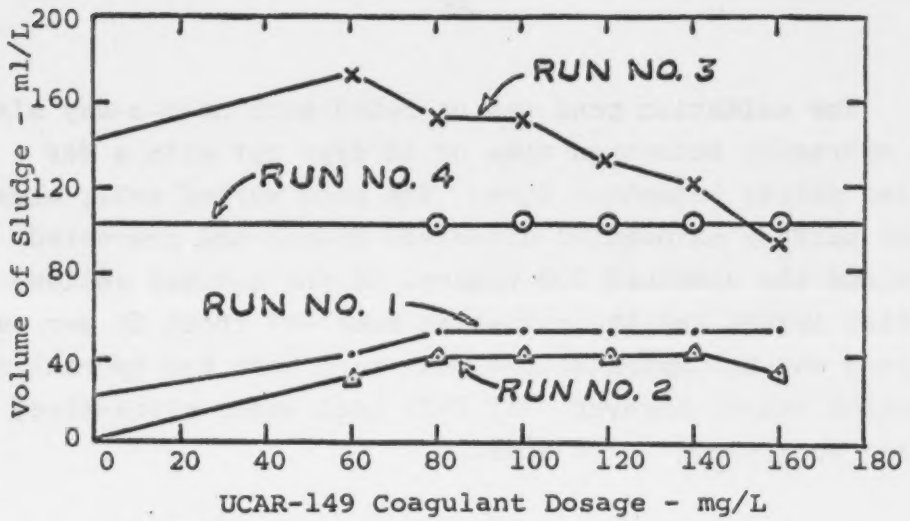


Figure 30 - CHANGE IN SUSPENDED SOLIDS IN SUPERNATANT WITH UCAR-149 COAGULANT



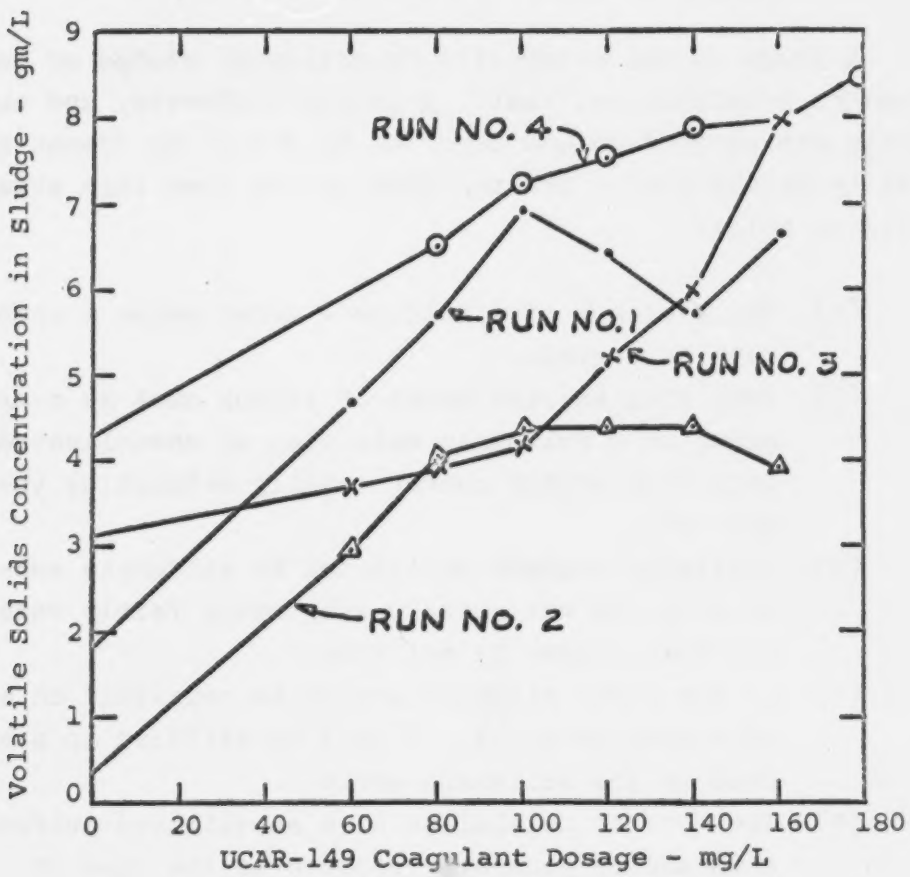


Figure 31 - VOLUME OF SLUDGE AND VOLATILE SOLIDS IN CONCENTRATED SLUDGE FORMED WITH UCAR-149 COAGULANT

The oxidation pond was operated with once-a-day mixing on a hydraulic detention time of 10 days but with a far greater solids retention time. The pond worked well; algae in the surface maintained dissolved oxygen and prevented odors, and the combined COD removal of the minimal solids aeration system and the oxidation pond was about 95 per cent. The pond was not operated for much more than two hydraulic detention times, however. If this idea seems attractive, further work needs to be done.

5. Metabolism of Sufonate Containing Organics

A study on the metabolism by activated sludge of benzene sulfonate, p-toluene sulfonate, p-phenol sulfonate, and sulfanilite was carried on and reported in detail by Symons and del Valle-Rivera (14). The ten conclusions from this study are listed below.

(1) The presence of a sulfonate group makes a compound hard to degrade.
(2) Even with an easy point of attack such as a hydroxy group on a sulfonate molecule, an unacclimated activated sludge cannot readily metabolize the molecule.
(3) Activated sludges acclimated to sulfonate substrates can use carboxy substrates fairly readily, but the reverse is not true.
(4) If any other point of attack is available on a sulfonate molecule, it will be utilized in preference to the sulfonate group.
(5) The ease of metabolism of a substituted sulfonate compound is generally related to the ease of attack on the substituted group, but spacial configuration also has an effect.
(6) Benzene sulfonate is metabolized through the sulfonate group.

(7) p-Toluene sulfonate is probably metabolized through the methyl group.

(8) p-Phenol sulfonate is metabolized through the hydroxy group.

(9) Sulfanilate showed bacterialstatic properties, but was metabolized through the amino group.

(10) As indicated by the p-amino benzoate metabolism, the presence of an amino group in an aromatic compound does not provide too easy a point of biological attack.

VII. CONCLUSIONS

1. Feeding schedule, batch or continuous, has a strong influence on the resultant solids produced.
2. Wasting method, batch or continuous, has little influence on the resultant solids produced.
3. When operating in the minimal solids aeration regime, substrate has a strong influence on the resultant solids produced by conventional activated sludge. This influence decreases as the solids retention time is increased.
4. When operating in the minimal or moderate solids aeration regime, substrate has an influence on the resultant solids produced by complete mixing activated sludge. This influence is not nearly as strong in a complete mixing activated sludge system as it is in a comparable conventional activated sludge system. Also, as above, the influence which is noticed at shorter solids retention times, decreases as the solids retention time lengthens.
5. The higher Mr/Fr ratios resulting from carbohydrate metabolism are due to more cells being produced, and is not due to cells having a higher percentage of biologically "hard" material.
6. Solids resulting from metabolism of mixed substrates at a given solids retention time can be calculated from a knowledge of the make-up of the mixture and the Mr/Fr ratios of the constituents at the same solids retention time and for the same loading schedule.
7. Volumetric organic loading has very little, if any, influence on the Mr/Fr ratio in either conventional or complete mixing activated sludge systems.
8. Highly volumetrically loaded, short solids retention time, complete mixing activated sludge systems offer the possibility of rapidly removing large quantities of COD from solution in a small aeration tank, if the problem in solids removal and final solids destruction can be solved.

9. An attempt to continuously waste mixed liquor from a continuously fed activated sludge pilot plant by discharging mixed liquor in a volume equal to the feed volume through a dosing syphon was not successful.
10. The influence of a varying solids retention time on the Mr/Fr ratio was similar both in conventional and complete mxing activated sludge.
11. In conventional activated sludge the new cell yield in mass units was estimated to be 0.6 to 0.7 of the COD removed while the inert mass produced in mass units was estimated to be 0.16 of the COD removed.
12. In complete mixing activated sludge the new cell yield in mass units was estimated to be about 0.5 of the COD removed, while the inert mass produced in mass units was estimated to be 0.09 of the COD removed.
13. For complete mixing activated sludge the rate of solids destruction under fed conditions was estimated to be 0.58 per day, which seemed high.
14. For conventional activated sludge, the rate of solids destruction under fed conditions was estimated to be 0.21 to 0.29 per day.
15. The Mr/Fr ratio increases, up to 25 per cent over normal operation, as the nitrogen fed to an activated sludge decreases.
16. The per cent nitrogen in the mixed liquor volatile solids declines as the COD/N ratio increases, so the quantity of nitrogen wasted per unit time is kept to about 80 per cent of the nitrogen added. Four per cent nitrogen is the lower limit of this decrease.
17. The nitrogen requirement of an activated sludge system is related to the Mr/Fr ratio.
18. The efficiency of removal of COD declines as the nitrogen content of the mixed liquor volatile solids goes down. This correlation is only slightly influenced by substrate and solids retention time, but is dependent on the type

of nitrogen used for supplementation.

19. The equations of the correlation mentioned in the above conclusion are:

NH_3-N Fed % COD Remaining = 142 $(\%N)^{-1.78}$

NO_3-N Fed % COD Remaining = 460 $(\%N)^{-1.95}$

NO_2-N Fed % COD Remaining = 280 $(\%N)^{-1.90}$

20. Nitrogen fixation was successful as a method of supplementation of a nitrogen-free organic feed, both for conventional activated sludge and complete mixing activated sludge, on a laboratory basis. Operation with a long solids retention time was beneficial.

21. Use of pure cultures and mixed pure cultures does not seem like a very good method of measuring the oxygen uptake of active mass.

22. Five completely, or nearly completely, active mass activated sludges gave an oxygen uptake rate of 14.6 mg/l/hr/1000 mg/l of volatile mass, with a standard deviation of 3.3 mg/l/hr/1000 mg/l of volatile mass. From this, an oxygen uptake rate of 15 mg/l/hr/1000 mg/l of active mass is suggested.

23. A formula for the calculation of total oxygen uptake of unfed active masses of the form $O_2 = KMao(1-e^{-kt})$ did not fit the data taken in these experiments.

24. Preliminary data indicates that a nearly completely active mass responds to high concentrations of glucose at an initial oxygen uptake rate of about 50 mg/l/hr/1000 mg/l of active mass.

25. An unfed die-away curve for mixed pure cultures gives a degradation rate of 0.32 per day and yields a final mass of 13 per cent of the original mass (Mdo/Mao = 0.87).

26. In one particular unfed, die-away experiment, the addition of a mixed culture of rotifers (predators) did not influence the behavior of a mixed pure culture.

27. The inert material which accumulates in a long SRT activated sludge is actually slowly biodegradable.
28. Use of an extended solids aeration complete mixing activated sludge system as a means of coagulating and destroying the solids discharged from a highly volumetrically loaded minimal solids aeration complete mixing activated sludge system was successful. The combined system provided high removal of organic matter and resulted in minimum solids production.
29. Chemical coagulation of the effluent from minimal solids aeration complete mixing activated sludge systems was successful, both with $Al_2(SO_4)_3$ and an organic coagulant. The high phosphate buffer concentration carried in the system caused high dosages, however.
30. Treatment of the minimal solids aeration complete mixing activated sludge system effluent in an oxidation pond seemed successful, but the pond was not operated for an extended period of time.
31. Biological metabolism of sulfonate containing organic molecules through attack of the sulfonate group is unlikely.

VIII. SUGGESTIONS FOR FUTURE WORK

1. More simple substrates should be studied at short solids retention times in complete mixing activated sludge and at long solids retention times in conventional activated sludge to determine more exactly the influence of substrate on the resultant solids production under these conditions.
2. The reason for the decline in Mr/Fr ratio in conventional activated sludge as the SRT is lowered below 1.5 to 2 days should be investigated.
3. The cause of the severe variations in mixed liquor volatile solids concentrations during continuous wasting through a dosing syphon should be studied.
4. Radioactive carbon studies should be made to accurately determine the new cell yield under conventional and complete mixing activated sludge conditions.
5. An attempt should be made to directly measure the rate of solids destruction under fed, or plant, operating conditions.
6. Studies should be made to determine the cause of the increase in the Mr/Fr ratio with decreasing quantities of nitrogen added to an activated sludge system.
7. Studies should be made over wide ranges of solids retention times and with several substrates to determine the minimum nitrogen requirements. Also, the nitrogen requirements under these conditions should be measured with nitrite and nitrate nitrogen as the supplemental form.
8. The influence of volumetric loading on the decline in efficiency of COD removal with declining nitrogen content of the mixed liquor volatile solids should be investigated.
9. The cause of the decline in efficiency of COD removal as an activated sludge becomes nitrogen-lean should be investigated.

10. A field study of nitrogen fixation as a mechanism of supplementing a nitrogen deficient industrial waste should be made.
11. Experimentally find and verify a formula expressing the oxygen uptake pattern with time of an active mass.
12. Find a reliable direct measure of the active mass in activated sludge.
13. Determine the die-away pattern of a nearly completely active activated sludge mass.
14. Determine, under activated sludge conditions, if the rate of solids destruction is influenced by the presence of external food.
15. Determine the maximum volumetric loading on a secondary system of a two-stage activated sludge unit which would be consistent with good overall operation.
16. Investigate the possibility of dewatering and incineration of chemically coagulated minimal solids aeration complete mixing activated sludge effluent. Also, obtain more reliable dosages with practical buffer levels.

IX. PROJECT PUBLICATIONS

1. Symons, J.M., and del Valle-Rivera, L.A., "Metabolism of Organic Sulfonates by Activated Sludge," Proceedings of the American Society of Civil Engineers, Journal of the Sanitary Engineering Division, 87, 53-70 (September 1961), Proceedings of the 16th Purdue Industrial Waste Conference, XLVI, 555-571 (1961), Transactions of the American Society of Civil Engineers, 127, 43-60 (Part III, 1962).

2. Symons, J.M., "Simple, Continuous-Flow, Low and Variable Rate Pump," Submitted to the Journal of the Water Pollution Control Federation, March, 1963.

3. Symons J.M., and Labonte, R., "Procedure for Continuous Nitrification Corrections During Warburg Respirometer Studies," Presented at the 18th Purdue Industrial Waste Conference, April 30, May 1, 2, 1963.

4. Tenney, M.W., Johnson, R.H.,Jr., and Symons, J.M., "Minimal Solids Aeration Activated Sludge," Presented at the 18th Purdue Industiral Waste Conference, April 30, May 1, 2, 1963.

5. Kaylor, F.B., Bechir, M.H., and Symons, J.M., "Atmospheric Nitrogen Fixation in Activated Sludge," Presented at the 18th Purdue Industrial Waste Conference, April 30, May 1, 2, 1963.

PLANNED PUBLICATIONS

1. Bechir, M.H., and Symons, J.M., "Influence of Nitrogen Deficiency on the Behavior of Complete Mixing Activated Sludge."

2. Symons, J.M., "Influence of Environmental Factors on the Solids Resulting from Activated Sludge Metabolism."

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1. Symons, J.M., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge," Unpublished Sc.D. Thesis, M.I.T., 1957. (Work part of N.I.H. Project E-1438).

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6. Kaylor, F.B., "Atmospheric Nitrogen Fixation in Activated Sludge," Unpublished Master's Thesis, Massachusetts Institute of Technology, May, 1961.

7. McKinney, R.E., and Symons, J.M., "Growth and Endogenous Metabolism, A Discussion," Presented at the International Conference on Water Pollution Research, London, England, September 3-7, 1962.

8. Kountz, R.R., and Forney, C., Jr., "Metabolic Energy Balances in a Total Oxidation Activated Sludge System," Sewage and Industrial Wastes, 31, 819-825 (1959).

9. McWhorter, T.R., and Heukelekian, H., "Growth and Endogenous Respiration Phases in the Oxidation of Glucose," Presented at the International Conference on Water Pollution Research, London, England, September 3-7, 1962.

10. Duncan, M.G., and Campbell, J.J.R., "Oxidative Assimilation of Glucose by Pseudomonas Aeraginosa, Journal of Bacteriology, 84, 784-792 (1962).

11. Norris, F.C., Campbell, J.J.R., and Ney, P.W., "The Intermediate Metabolism of Pseudomonas Aeroginosa,I. The Status of Endogenous Respiration," Canadian Journal of Research, 27, Section C, 157-164 (1949).

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15. Johnson, R.H., Jr., "Minimal Solids Aeration Complete Mixing Activated Sludge," Unpublished S.E. Thesis, Massachusetts Institute of Technology, June, 1963.